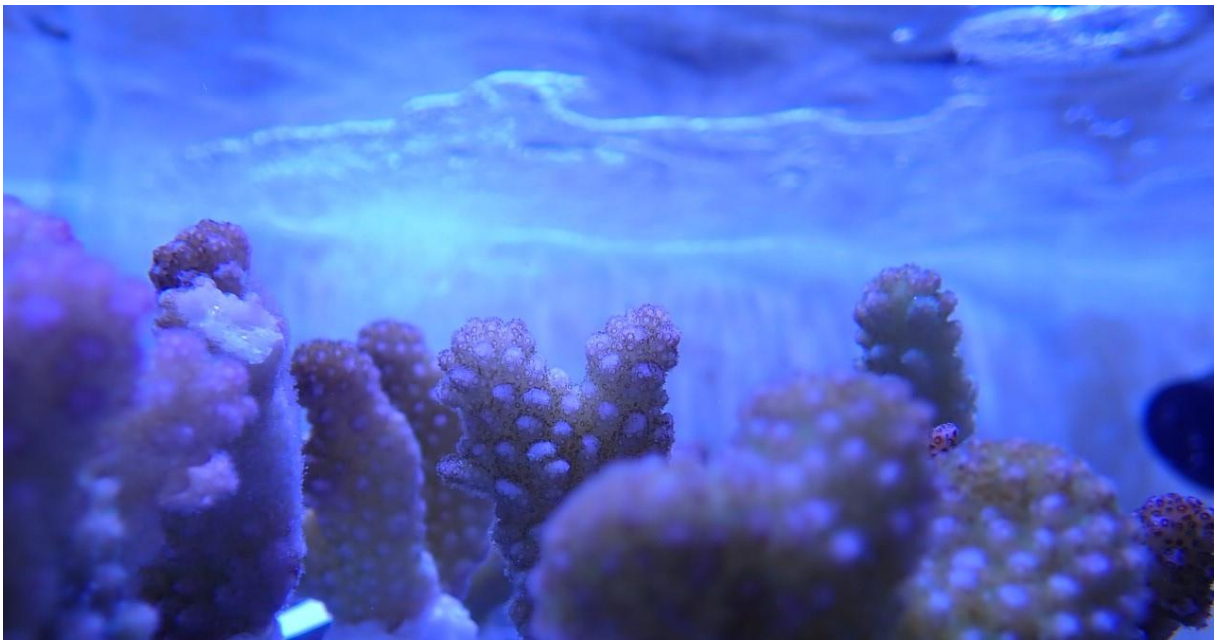
Transplantation of the microbiome from heat-tolerant to heat-sensitive corals – a new tool for building coral resilience to bleaching?



Master's Thesis in Biological Oceanography,

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1. Abstract

Tropical reef-building corals are acutely threatened by ocean warming which calls for active interventions that reduce further coral bleaching (i.e. the disruption of coral symbiosis with their unicellular photosymbionts) and subsequent coral mass mortality. Manipulation of fast-evolving bacterial communities associated with the coral host (i.e. the microbiome) might be one strategy to enhance coral resilience to bleaching. These communities are recognized as a vital part of the coral microbiome and can be important players in coral health and fitness, e.g. various bacterial functions could be beneficial for corals by potentially tuning the physiological response to heat stress. This study presents an experimental approach to develop a cost-effective method of coral microbiome transplantation aiming at building coral resilience to bleaching. Using a short-term heat stress assay, we identified heat-tolerant coral colonies of *Pocillopora spp.* that originate from a high variability habitat in the Thai Andaman Sea. Next, we used these corals as a source for bacterial microbiome consortia, which are supposed to promote bleaching resilience. Microbiome transplantation was conducted using a fresh tissue homogenate from these heat-tolerant corals and applying this inoculate on heat-sensitive conspecifics. Results indicate that recipient corals performed better under short-term acute heat stress compared to the control group. The bleaching response of inoculated corals was mitigated to a certain degree, but photosymbionts remained stressed within the holobiont. In a subsequent analysis of 16S rRNA amplicon data from this experiment we identified nine candidates of potentially transplanted bacterial taxa, comprising for instance the family Spongiibacteraceae which is known to be typically associated with coral holobionts. Additionally, two taxa were identified as *Bdellovibrio*, which are known *Vibrio* predators and might have played a role as pathogen antagonists preventing a *Vibrio* induced bleaching response in the experiment. These first results are preliminary and the role of potentially transplanted bacterial taxa in the mitigated stress response remains hypothetical and requires further studies.

2. Introduction

Many ecosystems around the world are already facing the consequences of global climate change, but possibly none as drastic as tropical coral reefs. This has demonstrated by the destructive force of recent global coral bleaching events (Hughes et al. 2018). Coral reefs are not only hotspots of biodiversity, they also provide fundamental ecosystem services to us humans, such as fisheries, coastal protection, and tourism (Moberg and Folke 1999). Rising ocean temperature and acidification are the major causes of the demise of this valuable and charismatic ecosystem (Hoegh-Guldberg 1998; Hughes et al. 2018). Tropical reef-building corals are affected by the slightest increases in temperature since they live near their thermal limits. Such temperature stress causes coral bleaching and mortality, which is mostly due to the breakdown of the obligate symbiosis between the coral animal and its photosynthetic dinoflagellate endosymbionts (Hoegh-Guldberg 1998). In general, reef-building corals host taxonomically and functionally diverse unicellular symbionts as part of their microbiome. Dinoflagellate photosymbionts of the family Symbiodiniaceae (LaJeunesse et al. 2018) which supply energy by processes like carbon fixation and translocation to the coral host (Muscatine and Porter 1977; Fig. 1) have been long known and are most important for the coral. In general, microorganisms on and within the tissues of many host organisms such as corals have been recognized as an integral part of the holobiont and can be of relevance for fitness (Lamberti et al. 2018). The unity of such holobiont associations, has established a new perspective on fitness and adaptation. Microbial roles in immunity, metabolic function (Thaiss et al. 2016), disease mitigation (Mao-Jones et al. 2010), and/or early development (Fraune & Bosch 2010) are known or hypothesized for various holobionts.

Current rates of ocean warming are too fast for reef-building corals to genetically adapt. This is mainly due to their relatively long generation times of approximately four to eight years (Reusch 2014; Webster and Reusch 2017). In this context, a realistic possibility to preserve these valuable ecosystems for future generations may be the idea of assisted evolution, which aims to accelerate evolutionary processes to enhance coral stress resilience (van Oppen et al. 2015). Some approaches of assisted evolution target selective breeding of corals or experimental evolution of the photosymbionts in the laboratory (van Oppen et al. 2015). Another promising strategy focuses on the communities of coral-associated prokaryotic microorganisms (Fragoso Ados Santos et al. 2015; Damjanovic et al. 2017; Fig. 1). In contrast to their long-lived coral host

populations, these fast-evolving associated bacteria seem to be auspicious, since they are hypothesized to promote physiological adaption even within one generation of the coral holobiont (van Oppen et al. 2015). Manipulation of the fast-evolving communities of the coral microbiome might be one way to buy more time to prevent further loss of reef-building corals to bleaching events, while working on the overall goal of tackling climate change.

Reef-building corals are known to host a vast diversity of bacteria (Blackall et al. 2015). Due to the high complexity of the coral holobiont, comprising complex and dynamic bacterial communities, photosymbiotic dinoflagellates, viruses, and fungi side by side, bacterial contributions are not yet well understood (reviewed in Epstein et al. 2019; Blackall et al. 2015; Fig. 1). Despite the growing body of studies investigating coral-associated bacterial microorganisms (in the following referred to as 'coral microbiome'), their contribution and interaction with the coral host still remains hypothetical (Bourne et al. 2016). However, metagenomic studies of bacterial functions in corals revealed capacities for nitrogen (Lesser et al. 2007) and carbon cycling (Neave et al. 2017), as well as degradation of aromatic compounds which could be beneficial for the host (Wegley et al. 2007; Thurber et al. 2009). The coral probiotic hypothesis assumes that coral microbiome communities are shaped through a dynamic interaction between the host and the environment, leading to a beneficial community which results in ecological success (Reshef et al. 2006; Fig. 1). Such changes of microbiome composition are either related to 'shuffling', i.e. abundance shifts of bacterial taxa (and their gene products) or to 'switching', i.e. the introduction of new taxa (and their genes) to the holobiont (Webster and Reusch 2017). Indeed, first studies of coral microbiomes have demonstrated that changes in their composition occurred in response to different environmental pressures such as pollution or ocean warming (Webster et al. 2016;

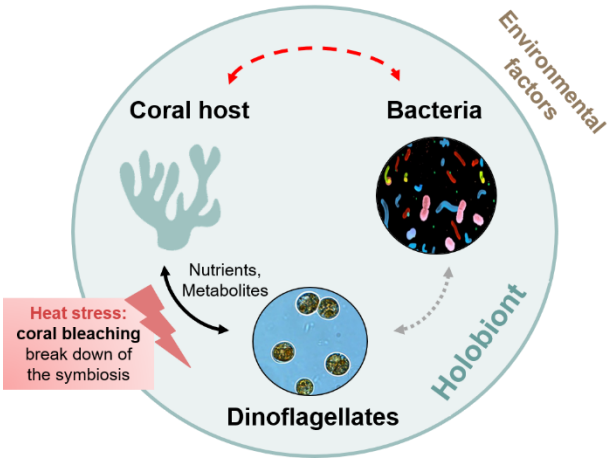

Figure 1 The coral holobiont consists of the coral host, its symbiotic partners, photosymbiotic dinoflagellates and associated bacteria, forming a dynamic network of balanced interactions which is influenced by environmental factors such as heat stress. Among other functions, bacteria are suggested to be involved in tuning the physiological response to heat stress and could therefore prevent coral bleaching (the breakdown of the coral-dinoflagellate symbiosis).

Ziegler et al. 2016). Moreover, it has been shown that coral microbiome composition aligns with heat tolerance of the coral host, suggesting that specific members of the microbiome may be involved in tuning the physiological responses to heat stress (Gilbert et al. 2012; Ziegler et al. 2017). A microbial process that could be involved in helping the coral tolerate heat stress is, among others, antioxidant activity which counteracts reactive oxygen species (ROS). These compounds are produced under stress inside the host and photosymbiotic dinoflagellate cells and are suggested to trigger the bleaching response that entails photosymbiont loss (Tchernov et al. 2004; Smith et al. 2005). Indeed, taxonomy-based functional profiling of corals exhibiting heat tolerance showed the enrichment of the protein ferredoxin NifW, a known scavenger of ROS (Ziegler et al. 2017). Consequently, the composition of the coral microbiome is likely to influence the trait of heat tolerance and the idea of bacterial community manipulation can be regarded as a potential and promising tool to enhance bleaching resilience in corals within the scope of assisted evolution.

There are two different approaches of microbiome manipulation (Mueller and Sachs 2015; Epstein et al. 2019). First, manipulative changes of the bacterial community can be achieved via direct selection, i.e. the identification of a specific beneficial microbe or microbial consortium based on bacterial functions and its introduction into the native microbiome of a host organism. Second, the approach of indirect selection requires the identification of a specific host phenotype which is supposed to carry microbiome functions of interest. This specific host and its indirectly selected microbiome serve as the source for beneficial taxa and consortia which can be introduced into other host organisms. Direct selection is being applied successfully in agriculture. It is of advantage because it targets specific beneficial bacterial functions and thus leads to more control over the applied manipulation (Epstein et al. 2019). For instance, the inoculation of rice plants, *Oryza sativa,* with a specific endophytic fungus increased the plants' growth rates (Redman et al. 2011). Also, it also has been shown that a specific trait such as heat tolerance of a holobiont could be conferred by microbiome manipulation. For instance, it was possible to enhance heat tolerance of aphids by inoculation with the symbiotic bacterial strain *Buchnera* (Moran and Yun 2015). Since direct selection requires previous knowledge of specific microbial functions that can only be acquired via culturing and meta'omics methods, indirect selection might be a more cost-effective and time-efficient method (Epstein et al. 2019). Indirect selection of microbiome function has been successfully applied in human medicine, when

inflammatory bowel disease (IBD) was treated with fecal microbiome transplantation (Borody and Khoruts 2012; Gupta et al. 2016). Here, beneficial microbiome functions were sourced from healthy donors and IBD was treated by transplanting fecal matter from healthy donors to diseased recipients (Borody and Khoruts 2012; Gupta et al. 2016).

Based on successful applications in agriculture and medicine, Peixoto et al. (2017) proposed the concept of beneficial microorganisms for corals (BMC), suggesting to make use of beneficial microbes to enhance coral stress tolerance, and in particular bleaching resilience, and incorporating such approaches into coral reef restoration efforts. The concept of BMC aims at direct selection of beneficial coral microbes by isolating them and screening for beneficial traits. Identified BMC then need to be applied to corals and tested in controlled experiments under environmental stress conditions (Peixoto et al. 2017). To date, manipulation of the coral microbiome to enhance stress tolerance has been tested in preliminary studies (Fragoso Ados Santos et al. 2015; Rosado et al. 2018). Indeed, a first proof of concept study has demonstrated potential success of the BMC concept (Rosado et al. 2018). Bacterial isolates from *Pocillopora damicornis* were screened for antagonistic activity against the coral pathogen *Vibrio coralliilyticus* (Ben-Haim et al. 2003), ROS degrading enzymes, sulfur cycling (i.e. degradation of dimethyl sulfoniopropionate (DMSP)), and nitrogen cycling (Rosado et al. 2018). An inoculation with the selected BMC successfully reduced signs of bleaching in corals subsequently exposed to a heat and pathogen treatment. This study provided first confirmation that the coral microbiome can be manipulated to mitigate coral bleaching effects caused by a pathogen (Rosado et al. 2018). Usually, *V. coralliilyticus* has been shown to decrease coral health by triggering bleaching responses (Ben-haim et al. 2003).

The overall objective of this study is to test a new cost-effective method of coral microbiome manipulation based on the approach of indirect selection. This study tests a simple and affordable approach that will be likely to find application in coral reef restoration programs worldwide, including programs that do not receive sufficient financial funding. The approach intends to source microbiomes from heat tolerant coral phenotypes that likely promote bleaching resilience of their hosts. Compared to direct selection approaches, no prior investment on identifying bacterial function via microbiological cultivation methods and/or meta'omics is considered. Even though it has been proposed in assisted evolution objectives that selecting for a heat-tolerant

phenotype includes time-consuming multigenerational coral experiments using a selection pressure (i.e. increased temperature; van Oppen et al. 2015; Epstein et al. 2019), there are means to bypass *ex situ* cultivation. This study makes use of corals which had acquired a stress tolerant phenotype due to natural exposure to environmental selection pressures (i.e. in high variability environments) over multiple generations. These coral populations offer biological material of microbiomes that are potentially beneficial to corals under environmental stress.

Accordingly, this study utilizes a unique coral reef system in the Andaman Sea off the coast of Thailand. Coral populations on the west shore reefs of several islands in the Andaman Sea are exposed to large-amplitude internal waves (LAIW/solitons) that carry nutrient-rich and cold deep sea-water, causing strong fluctuations in temperature and nutrients (Leichter et al. 1996; Jantzen et al. 2012; Wall et al. 2012; Fig. 2). It has been suggested in earlier studies that corals located on the exposed west shores of the islands exhibit higher heat tolerance than their conspecifics from the sheltered east shores (Buerger et al. 2015). Following up on previous findings, this study aims to (1) identify heat-tolerant coral colonies in the Thai Andaman Sea by using a short-term heat stress assay and (2) test a microbiome transplantation procedure using fresh tissue homogenate for the transfer of bacteria from these heat-tolerant corals to their heat-sensitive conspecifics.

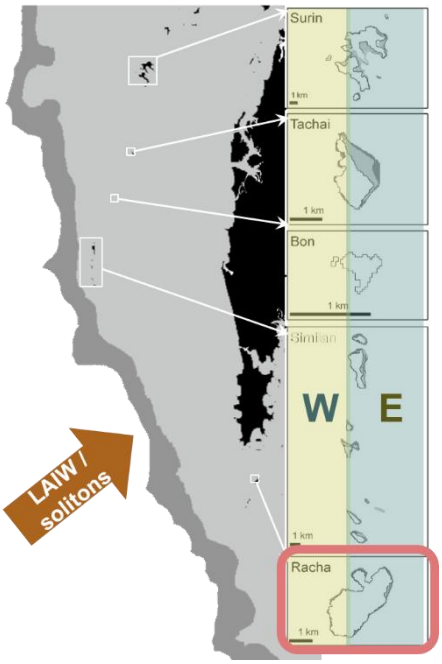

Figure 2 Island reefs in the Andaman Sea in Thailand are exposed to large-amplitude internal waves (LAIW/solitons) that carry deep water to the shallow reefs (up to 10 – 5 m) causing strong fluctuations in temperature and nutrient levels. This study used coral fragments from the east (E) a west (W) shore reefs of Racha Island (framed in red). (Adapted from © L Fillinger)

3. Methods

3.1 Study organism

The branching coral *Pocillopora spp.* was selected as a study organism because of its ecological importance as one of the most abundant reef builders in the entire Indo Pacific (Phongsuwan and Chansang 1992; Yeemin et al. 2009). *P. spp.* has been used in multiple experimental studies and genome data of *Pocillopora damicornis* has been

generated recently (Traylor-Knowles et al. 2018). For this study we selected the *Pocillopora verrucosa* species type by visual identification. In the Andaman Sea this type is particularly known to dominate reefs that receive increased wave exposure (Brown 2007).

3.2 Heat stress assay

3.2.1 Aquarium and treatment set-up

Short-term heat stress assays were conducted to compare the phenotypic and physiological responses of coral colonies originating from the east and west shores of Racha Island, in order to identify their relative heat tolerance. One month before the start of the experiment, temperature, a proxy for soliton impact, was monitored continuously using a temperature logger per reef site (HOBO Pendant Temperature/Light 8K Data Logger, Onset, USA; Fig 3A). Subsequently, colonies of *Pocillopora spp.* were sampled from the eastern (RE; N = 10; 15 m*;* 7.598910 N, 98.373100 E) and western shore of Racha Island (RW; N = 9; 15 m; 7.595530 N, 98.354320 E) at the end of March 2018. Two coral fragments (5 cm) were collected per colony using a chisel and a hammer and were transported inside aerated seawater aquaria within 2 hours to the coral facilities at Phuket Marine Biological Center (PMBC, Cape Panwa, Phuket, Thailand). Each fragment was fixed with cable ties to a PVC ring (Ø 1 inch) and acclimated for 12 days in an aquarium to minimize sampling effects (Fig. 3 B). During the acclimation period, fragments were maintained in a 500 L flow-through tank with a flow rate of 3.6 L/min and water temperature of 29.4 ± 0.3 °C. To mimic light conditions of the sampling sites (Jantzen et al. 2012), LED lamps (135 W, Hydra Fiftytwo HD LED, Aqua Illumination, USA) created a 12/12 hour light/dark cycle with an average light intensity of 74.54 ± 5.46 µmol m^{-2} s^{-2}. Tank conditions throughout the experiment were constant with 7.56 ± 0.03 mg/L in dissolved oxygen and a salinity of 32.6 ± 0.2 ppt.

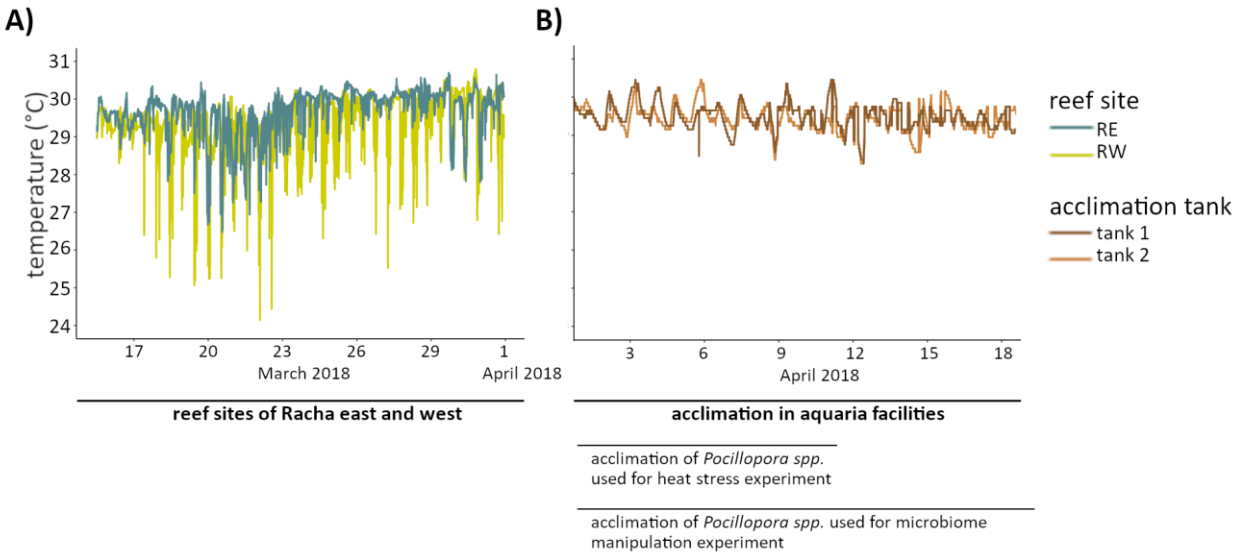

Figure 3 A) Seawater temperature (°C) at the respective study sites Racha west (RW) and Racha east (RE) during March 2018. The colonies of *Pocillopora spp*. were exposed to 29.6 ± 0.6 °C at RE and to 29.3 ± 0.8 °C at RW. B) Seawater temperature (°C) per tank during the acclimation period of the collected *Pocillopora spp*. fragments in April 2018. Coral fragments for the heat stress assay were maintained at 29.4 ± 0.3 °C for 12 days and fragments used for the microbiome transplantation experiment for 24 days.

The heat stress assay was carried out from 11th until 13th March 2018. Two experimental tanks (40L, N = 4) were maintained inside a 500 L flow-through water bath, for temperature control. Water baths were connected to a 500 L source tank which was supplied with 5 µm-filtered seawater from the reef adjacent to PMBC. The source tank was held constant at 29.4 ± 0.3 °C using a chiller and a heater connected to a temperature controlling device (Titanium Heater 100 W, Schego, Germany; Temperature Switch TS 125, HTRONIC, Germany; Aqua Medic Titan 1500 Chiller, Germany). Seawater of the experimental tanks was changed manually once a day using water from the source tank. Each experimental tank contained a heater connected to a temperature-controlling device, a temperature logger, air supply and one small current pump (Titanium Heater 100 W, Schego, Germany; Temperature Switch TS 125, HTRONIC, Germany; HOBO Pendant Temperature/Light 8K Data Logger, Onset, USA; Koralia nano 900 L/h, Hydor, Italy). Coral fragments were randomly distributed among the experiment tanks and treatments, resulting in one fragment from an individual colony per experimental tank. Overall, one experimental tank consisted of 9 to 10 coral fragments, respectively (5 from RE, 4-5 from RW).

Duplicate heat treatment tanks were established. The heat treatment (34 °C) consisted of increasing temperatures from 29°C to 34°C over 4 h, held at 34 °C for 6 h and decreasing temperatures to 29 °C within 4 h which were then maintained for 16 h. The

duplicated ambient treatment (29°C) was held at a constant water temperature of 29 °C over the duration of the experiment for 24 h (Fig. 5).

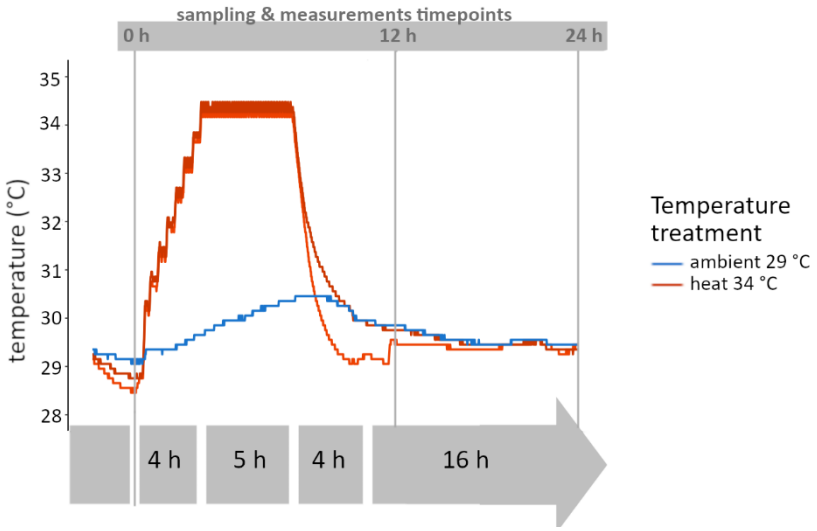

Figure 4 Temperature profiles (°C) were measured in the two duplicated treatments, i.e. heat and ambient, over the duration of the heat stress assay. Measured average water temperatures were 30.8 ± 2.0 °C in the heat treatment and 29.7 ± 0.3 °C in the ambient treatment over the course of the experiment. Measurements associated with coral health (bleaching score and effective quantum yield) were taken at the beginning of the experiment at 0 h, after 12 h and after 24 h.

3.2.2 Quantification of coral fitness parameters

A bleaching score was recorded as a measure of bleaching state and photosymbiont density (Siebeck et al. 2006). Measurements were performed at three time points for each fragment, before (1) start of experiment at 0 h, (2) after 12 h, and (3) at the end of experiment at 24 h (Fig. 4). The bleaching score was visually categorized by one observer on a scale from 1 (completely bleached) to 6 (healthy) by using the 'Coral Health Chart' (Siebeck et al. 2006; Coral Watch, reefquest.org) (Fig. 5).

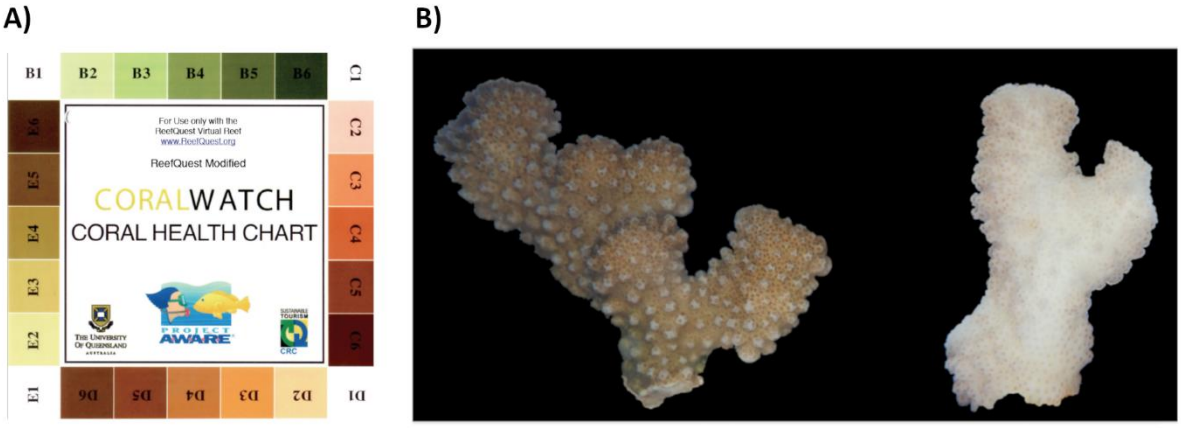

Figure 5 A) Levels of bleaching score were visually categorized by using the 'Coral Health Chart' (Coral Watch, reefquest.org). B) Phenotypic responses of *Pocillopora spp*. fragments subjected to the heat 34 °C treatment showing different levels of tissue condition. The fragment on the left can be visually categorized as 5 (healthy) and the fragment on the right as 1 (completely bleached).

The photosynthetic efficiency of the dinoflagellate photosymbionts was assessed as the effective quantum yield by using a pulse amplitude-modulated fluorometer (Diving-PAM, Walz, Germany). This parameter indicates the efficiency of the photosystem II (PSII) in ambient light adapted conditions (Ralph and Gademann 2005) and is estimated as follows: Φ PSII = (Fm' – F) / Fm' = ΔF / Fm' (Genty et al. 1989). Measurements of light adapted coral fragments were taken at the beginning of the experiment at 0 h, after 12 h and at the end of the experiment at 24 h (Fig. 4). To guarantee reproducibility between measurements, a spacer (transparent hose) kept a constant distance of 8 mm between coral fragment surface and the fiberoptics of the PAM. The main PAM settings were Measuring Light Intensity (MI) = 5 and Gain (G) = 3.

3.3 Microbiome transplantation experiment

3.3.1 Production of inoculation and control treatment

Two coral fragments were collected from three *Pocillopora spp.* colonies (N = 3) at 15 m in RW at the end of March 2018. These donor fragments were acclimatized for 24 days under the same conditions as described in *3.2.1* (Fig. 3 B). Subsequently, they were used to produce the coral tissue homogenate for the inoculation treatment at the start of the microbiome transplantation experiment, which was carried out from 22nd until 25th April 2018. To transplant members of the microbiome from RW to RE corals, an inoculation method which has been previously used to transmit coral disease from Gignoux-Wolfsohn (2012) was adopted. A homogenate of fresh coral tissue was produced by placing each donor fragment into one sterile 50 mL falcon tube with 15 mL of 0.2 µm filtered sea water and sterile glass beads (ø 2.7 mm; Gignoux-Wolfsohn et al. 2012, 2017) . Each falcon tube was vortexed (neoLab Vortex Genie 2, Germany) for one minute until no tissue remained on the skeleton (Kline & Vollmer, 2011). After removing the glass beads and the skeleton fragments, the coral tissue homogenates were merged into one pool of donor inoculate for the experiment, which was then distributed among ten 15 mL falcon tubes by adding 8 mL of the inoculate into each one. Triplicate samples of 2 mL of the inoculate were shock frozen for DNA extraction. The same procedure was carried out for the production of the control inoculate, i.e. vortexing six 50 mL sterile falcon tubes for one minute, each filled with 15 mL 0.2 µL filtered sea water (FSW) and sterile glass beads, without adding donor fragments. The

control inoculate was also mixed and ten 15 mL sterile falcon tubes were filled with respectively 8 mL.

3.3.2 Aquarium and treatment set-up

Four fragments (5 cm) each from five visually healthy colonies of *Pocillopora spp*. were collected from RE at a depth of 15 m at the end of March 2018. These recipient fragments (N = 5) were brought to the aquaria facilities at PMBC and were kept for 24 days in 500 L flow-through tanks under conditions as described in *3.2.1* (Fig. 3 B). The microbiome transplantation experiment consisted of two parts: (1) the inoculation experiment was conducted during the first 24 h and was followed by (2) a heat stress assay ending after another 24 h, resulting in a total of 48 h (Fig. 6). The microbiome transplantation experiment was carried out from 23rd until 25th March 2018. During both parts of the experiment, two 40 L experimental tanks were placed in 500 L flow-through water baths, respectively, to maintain water temperatures. The water baths were connected to a 500 L source tank which was supplied with 5 µm-filtered seawater from the reef adjacent to PMBC. The source tank was held constant at 29.4 ± 0.3 °C using a chiller and a heater connected to a temperature-controlling device (Titanium Heater 100 W, Schego, Germany; Temperature Switch TS 125, HTRONIC, Germany; Aqua Medic Titan 1500 Chiller, Germany). Each experimental tank contained a heater connected to a temperature controlling device, a temperature logger, air supply and one small current pump (Titanium Heater 100 W, Schego, Germany; Temperature Switch TS 125, HTRONIC, Germany; HOBO Pendant Temperature/Light 8K Data Logger, Onset, USA; Koralia nano 900 L/h, Hydor, Italy). At the start of the microbiome transplantation experiment, five coral RE fragments (one fragment from each colony) were distributed among all of the four experimental aquaria.

During the inoculation experiment two tanks per treatment (inoculation (I) and control treatment (*C*)) were established while seawater temperatures were constant at ambient 29 °C (Fig. 6). At the beginning, current pumps and aeration were interrupted and the seawater volume in all four experimental tanks was reduced to 8 L (to a water level of 6 cm). Subsequently, PVC tubes (7 cm height, 8 cm diameter, volume of 350 ml) were placed around each coral fragment to create a semi-enclosed microenvironment (Fuess et al. 2017). The treatment was carried out by adding either 8 ml of the donor inoculate or the control inoculate (0.2 µl as described in *3.3.1*) into the PVC tubes, respectively. The volume proportion of the inoculates added was 2.3

% of the volume of each PVC tube. Coral fragments were incubated inside the tubes for 30 minutes, providing an opportunity for uptake of bacteria. Afterwards, all PVC tubes were removed, flow and aeration was switched on, and water volumes inside experimental tanks were increased to 40 L by adding water from the source tank. The volume proportion of the inoculates was diluted to 0.1 % of the total volume of the experimental tank for the next 24 h. Tissue samples (one from each fragment at each time point) were collected before at 0 h and after the inoculation experiment at 24 h (Fig. 5). All samples for DNA extraction were collected using sterile clippers (1 - 2 cm of each fragment). Samples were rinsed with 0.2 µL FSW, wrapped in multiple sheets of sterile aluminum foil, crushed with a hammer, filled into a sterile 2 mL vial, and flash frozen in liquid nitrogen. Moreover, seawater samples (1 L) were taken from each of the experimental tanks at 0 h and 24 h of the inoculation experiment (Fig. 6). Seawater samples were vacuum-filtered over a 0.22 µl filter (Durapore PVDF filter membranes, Merck, Germany). Filters were immediately stored in a 2 mL cyro vial and flash frozen.

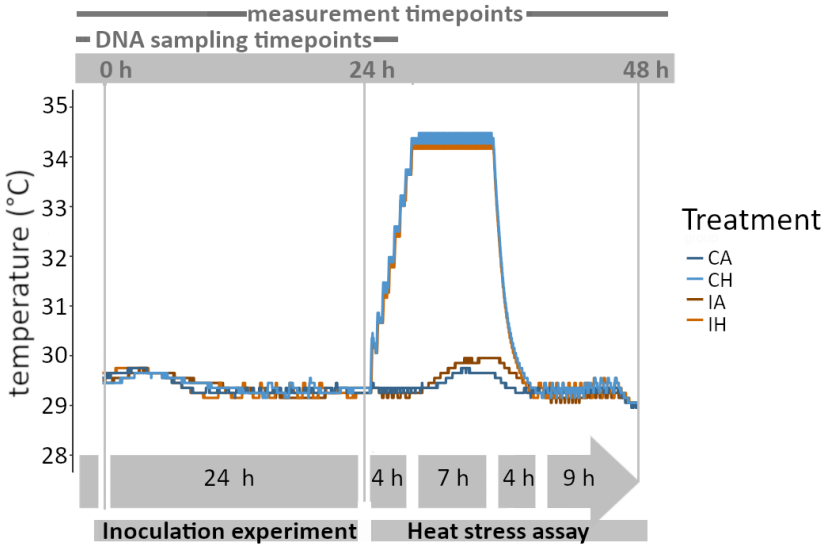

Figure 6 Temperature profiles (°C) measured over the course of the microbiome transplantation experiment. The experiment consisted of two parts: (1) the inoculation experiment, conducted during the first 24 h and (2) the heat stress assay, carried out until 48 h of the experiment. Coral and seawater samples (DNA samples) were taken at 0 h and 24 h, while fitness measurements (bleaching score and effective quantum yield) were taken at 0 h, 24 h and 48 h. Each line represents one treatment tank (Inoculation 29 °C (IA), Inoculation 34 °C (IH), Control 29 °C (CA) and Control 34 °C (CH)).

Table 1 Experimental design and treatment groups of the inociulation experiment which was part of the microbiome transplantation experiment.

Experiment	timepoint of sampling	Treatment	DNA sample type	DNA sample code	Coral sample name
Inoculation experiment	0 h	None	Fresh tissue homogenate	Tissue inoculate	Donor inoculate
	0 h	None	Coral	preI	Recipient coral fragments before inoculation
	24 h	Inoculation	Coral	I_postI	Recipient coral fragments after inoculation
	24 h	Control	Coral	C_postI	Control recipient coral fragments after inoculation

Before starting the second experiment part, a 50 % water change was conducted resulting in a further dilution of the inoculate (0.05 %) in the experimental tanks used during the first part. The heat stress assay consisted of the tanks IA, IH, CA, and CH, representing all treatment combinations of inoculation (I) or control (C) and ambient (A) or heat (H) temperature (Table 2; Fig. 6). By this means, the heat stress assay aimed at comparing the fitness responses of inoculated versus control coral fragments under heat stress. Both heat and ambient treatments were conducted following the same protocol as in chapter *3.2.1*. The heat treatment (34 °C) included an increase from 29°C to 34°C over 4 h, constant 34 °C for 7 h and a decrease to 29 °C within 4 h which was then maintained for 9 h.

Table 2 Overview of treatment group formation and names of the heat stress assay as part of the microbiome transplantation experiment.

Experiment	Inoculation experiment 0 h – 24 h	Heat stress assay 24 h – 48 h	Treatment group name
Heat stress assay	Inoculation	Heat 34 °C	Inoculation 34 °C = IH
	Inoculation	Ambient 29 °C	Inoculation 29 °C = IA
	Control	Heat 34 °C	Control 34 °C = CH
	Control	Ambient 29 °C	Control 29 °C = CA

3.3.3 Quantification of coral fitness parameters

Coral fitness responses were assessed as described in chapter *3.2.2*. Bleaching score and the effective quantum yield were recorded at three timepoints (1) before experiment start at 0 h, (2), at the end of the inoculation experiment at 24 h, and (3) at the end of the heat stress assay at 48 h (Fig. 6).

3.3.4 DNA extraction and 16S rRNA gene sequencing

All coral tissue samples were stored at -80 °C until DNA extraction using a modified protocol for the Qiagen Allprep DNA/RNA extraction kit. In the lab, samples were transferred into lysis tubes (2 mL Lysing Matrix E, MP Biomedicals, USA) filled with 800 µl RLT lysis buffer (Buffer RLT, Qiagen AllPrep Kit, Germany) and disrupted in a bead mill (2 x 1 min 30 Hz, Qiagen TissueLyser II, Germany). 400 µL of the sample was transferred to a 1.5 mL vial and centrifuged at 15,000 rcf for 3 minutes. The supernatant was processed following the manufacturer's protocol using DNA columns included in the kit (Qiagen AllPrep Kit, Germany). To promote cell lysis of seawater samples, frozen membrane filters were thawed for 5 min and frozen again at -20 °C for 5 min, repeating the cycle 3 times. Subsequently, filters were cut into half using sterile scalpels. One half was further sliced into stripes, transferred into a lysis tube (2 mL Lysing Matrix E, MP Biomedicals, USA) filled with 800 µl RLT lysis buffer (Buffer RLT, Qiagen AllPrep Kit, Germany), and further processed in parallel with the coral samples. A blank control sample was added at every second DNA extraction session. DNA was eluted from the columns using 50 µL 10 mM Tris-HCl (Buffer EB, Qiagen AllPrep Kit, Germany). DNA concentrations were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

The bacterial 16S rRNA gene was amplified in a one-step PCR approach (Hoellen et al. 2018). Primer constructs targeting the V5/V6 hypervariable region contained the 784F [5'AGGATTAGATACCCTGGTA '3] and 1061R [5'CRRCACGAGCTGACGAC'3] sequences (Nam et al. 2011) unique barcodes (Kozich et al. 2013), heterogeneity spacers, and linker sequences for paired end sequencing on the Illumina® MiSeq sequencer. In order to conduct PCR amplification of the bacterial 16S rRNA gene, we used approximately 10 - 30 ng of coral DNA and 5 - 15 ng DNA from seawater samples. PCR reactions were performed using 10 µL Phusion® High-Fidelity PCR Master Mix with HF Buffer (New England Biolabs, USA) and a final concentration of 0.5 µM for each primer in a total reaction volume of 20 µL. The amplification cycling temperatures consisted of one cycle at 98°C for 30 s, 30 cycles each at 98°C for 10 sec, 52°C for 30 sec, and 72°C for 30 sec, followed by a final extension step at 72°C for 5 min. Negative controls were included to verify reagents were uncontaminated (DNA extraction blanks and PCR blanks). A mock community was used as a positive control (#ZRC 190811, ZymoBIOMICS Microbial Community DNA, Zymo Reseach).

Amplicons were quantified via 2% agarose gel electrophoresis. Concentration of each sample was determined using the Quick-Load® Purple 100 bp DNA Ladder (New England Biolabs, USA) and Image Lab™ 6.0.1 (Software Life Science Research, Bio-Rad). Equimolar amounts of 18 - 24 samples were pooled into subpools, which subsequently were purified from an 1% agarose gel using the MinElute Gel Extraction Kit (Qiagen). After the quantification of the purified subpools by Qubit, equimolar amounts were pooled into one final pool that was stored at -20°C until sequencing. Sequencing was performed on a MiSeq sequencer (Illumina®) at the Norwegian Sequencing Centre (www.sequencing.uio.no, Oslo, Norway) using the MiSeq Reagent Kit v3 and 10% PhiX.

3.3.5 16S rRNA gene amplicon sequence processing

MOTHUR software (http://mothur.org/, version 1.39.5; Schloss 2009, 2011) was used for amplicon analysis. Raw sequences were split according to barcodes and assembled into contigs. Singletons (n = 1 over all samples) and rare sequences (n < 10 over all samples) were removed both by using the command *remove.rare()*. Unique sequence reads were merged using *unique.seqs()* and aligned against the SILVA alignment database (release n. 132; Quast 2013). Sequences that did not cover the hypervariable region V5/6 were removed (SILVA alignment position 23440 to 344151; Quast 2013). Moreover, sequences were pre-clustered (2 bp difference; Schloss 2011) and chimeric sequences were identified using *chimera.vsearch()* and removed using *remove.seqs()*. To classify the sequences, the *classify.seqs()* function in MOTHUR was used against the SILVA RNA gene database (80% bootstrap; release n. 132; Quast 2013). Afterwards non-targeted sequences (e.g. chloroplasts, mitochondria, archaea, eukaryotes, and unknown) were removed. To obtain operational taxonomic units (OTUs), the sequences were clustered at the 0.03 difference level. Data was normalized by subsampling to 4423 sequences per sample.

3.4 Statistical analyses

3.4.1 Coral fitness parameters

3.4.1.1 Bleaching score

Visualization and analyses of coral fitness were conducted in the statistical environment *R* (R Development Core Team, 2011, Version 3.2.2). First, proportions of the bleaching score counts were visualized in stack bar plots using the *ggplot2* library.

Data were grouped per treatment and time point. Next, we analysd the changes in bleaching score over time. Δ-bleaching score was calculated for each experiment and fragment by subtracting the final score from the initial score (Table 3). In order to test the one-tailed hypotheses for each experiment and experimental part (Table 3), a randomization test of 10,000 bootstraps was conducted. We applied a *for (i in 1:10000){}* loop in R on the mean difference (Δ-mean) between the respective treatments for either site (RW and RE) or inoculation treatment (I and C; Table 3). The resulting 10,000 Δ-means were plotted per site or inoculation treatment and a *P*-value was calculated each by dividing the proportion of values smaller than the true Δ-mean by the number of bootstraps.

Table 3 Calculation of Δ-bleaching score and Δ-mean, and hypothesis of the conducted randomization test per experiment and experimental part.

Experiment	Experimental part	Calculation of Δ-bleaching score	Calculation of Δ-mean	Hypothesis of randomization test
Heat stress assay		Δ-bleaching score = bleaching score at 24 h – bleaching score at 0 h	Δ-mean (RE or RW) = mean Δ-bleaching score $_{heat}$ – mean Δ-bleaching score $_{ambient}$	RW Δ-bleaching score $_{heat}$ = RW Δ-bleaching score $_{ambient}$ and RE Δ-bleaching score $_{heat}$ > RE Δ-bleaching score $_{ambient}$
Microbiome transplantation experiment	Inoculation experiment	Δ-bleaching score = bleaching score at 24 h – bleaching score at 0 h	Δ-mean = mean Δ-bleaching score $_{inoculation}$ – mean Δ-bleaching score $_{control}$	Δ-bleaching score $_{inoculation}$ = Δ-bleaching score $_{control}$
	Heat stress assay	Δ-bleaching score = bleaching score at 48 h – bleaching score at 24 h	Δ-mean (C or I) = mean Δ-bleaching score $_{heat}$ – mean Δ-bleaching score $_{ambient}$	I Δ-bleaching score $_{heat}$ = I Δ-bleaching score $_{ambient}$ and C Δ-bleaching score $_{heat}$ > C Δ -bleaching score $_{ambient}$

3.4.1.2 Effective quantum yield (ΔF/Fm)

Raw data of the photosynthetic efficiency measured as effective quantum yield (ΔF/Fm) were plotted per treatment over the time points. To quantify the changes of effective quantum yield (ΔF/Fm) over time, Δ-effective quantum yield was calculated respectively (Table 4).

Table 4 Calculation of Δ-effective quantum yield per experiment and experimental part.

Experiment	Experimental part	Calculation of Δ-effective quantum yield
Heat stress assay		Δ-effective quantum yield = yield at 24 h – yield at 0 h
Microbiome transplantation experiment	Inoculation experiment	Δ-effective quantum yield = yield at 24 h – yield at 0 h
	Heat stress assay	Δ-effective quantum yield = yield at 48 h – yield at 24 h

Differences of all Δ-effective quantum yield data were analysed using mixed effect models (LMEs, model with multiple random factors, R package *nlme*), where data met assumptions of normality (Shapiro-Wilk test). Otherwise, generalized linear mixed effect models were applied (GLMMs, *glmer* function, R package *lme4).* Models tested for the effect of island site (RE and RW), heat stress treatment (heat and ambient), and the interaction effect (site x heat stress treatment). The first part of the microbiome transplantation experiment data was tested by LME for the effect of the inoculation treatments (C and I) included as a fixed factor. In the second part of the experiment, GLMMs tested for the effect of the inoculation treatment (C and I), the heat stress treatment (29 °C and 34 °C), and the interaction effect (inoculation treatment x heat treatment). All GLMMs and LMMs were fitted by using the tank as a random factor. *P*-values for normally distributed data were reported by performing an ANOVA of the respective model. In the other cases *P*-values were reported by using Wald Statistics. Tukey multiple pairwise comparison post-hoc tests followed on the fitted models (LMEs and GLMMs) using the *lsmeans* function adjusted to *tukey* from the R package *lsmeans*.

3.4.2 16S rRNA gene amplicon sequence data

All analyses were performed using a dataset subsampled to 4423 sequences per sample as implemented in the R environment (Version 3.2.2, R Development Core Team, 2011) and MOTHUR (http://mothur.org/, version 1.39.5; Schloss 2009, 2011). Species richness (Chao 1 index was corrected for sample size) and species diversity (Shannon Index) were estimated with the *summary.single()* function (MOTHUR). These α-diversity indices were analysed each by comparing sample groups fitted as fixed factors using generalized linear mixed effect models (GLMMs, *glmer* function, R package lme4). Subsequent Tukey multiple pairwise comparison post-hoc tests on the fitted models were conducted by using the *lsmeans* function (R package lsmeans).

OTU abundance data was visualized using analysis of principal coordinates (PCoA).The Jaccard index dissimilarity matrix of presence absence data was generated (MOTHUR *pcoa* function) and plotted (*pcoa* function, R package *ape,* Fig. S2). Statistical differences in community structure between treatment groups were tested by analysis of similarity (ANOSIM; 9,999 permutations, package vegan). Venn diagrams and shared OTU tables were generated (*venn* function in MOTHUR) which provide unique, shared, and ubiquitous OTUs across samples grouped by treatments.

OTUs that are only shared between donor and recipient samples represent taxa potentially transplanted bacteria from the donor to the recipient coral fragments. Representative sequences (MOTHUR, get.OTUrep) of these first candidate OTUs were aligned in GenBank (NBCI National Center of Biotechnology Information) using BLASTn (Basic Local Alignment Search Tool; Altschul et al. 1990).

4. Results

4.1 Heat stress assay

4.1.1 Bleaching score

Results show that bleaching intensity of coral fragments from Racha East (RE) was significantly higher than of fragments from Racha West (RW) under the same acute short-term heat stress. Stack bar plots demonstrated a strong increase of the “completely bleached” phenotype category in the heat treatment among RE fragments (resulting in 45 % of bleaching score 1 fragments at 24 h, Fig. 7 A and B). In contrast, frequencies of the bleaching score categories of RW fragments did not change significantly over time in neither treatment (Fig. 7 C and D).

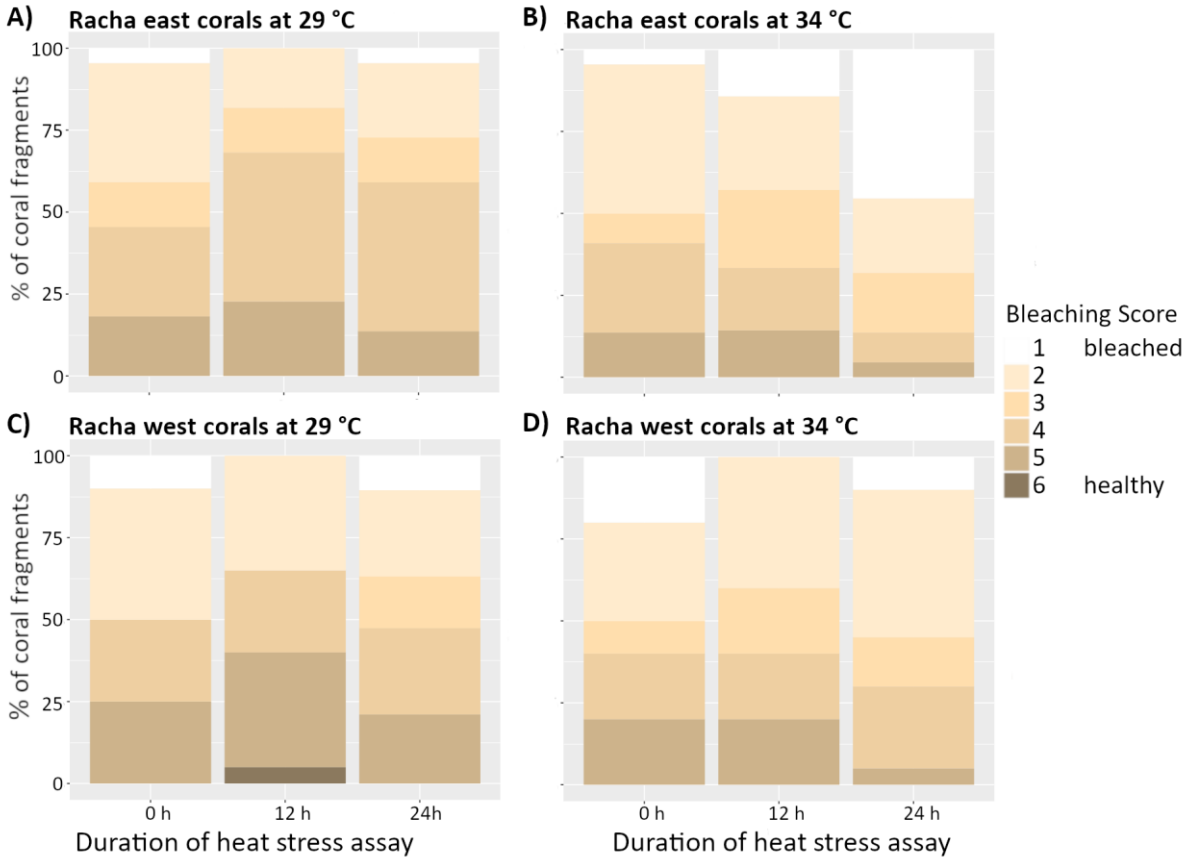

Figure 7 Bleaching score over the duration of the heat stress assay (heat = 34 °C, ambient = 29 °C) comparing the response between coral *Pocillopora spp.* fragments from Racha west (RW) and east (RE). The bleaching score was recorded at the beginning at 0 h, after 12 h and at the end of the experiment after 24 h by categorizing fragments on a scale from 1 (completely bleached) to 6 (healthy).

A certain decrease in Δ-bleaching scores can be observed for all coral fragments from both reef sites, RE and RW, exposed to heat (Fig. 7 B and D). However, the decrease of the Δ-bleaching score for RW fragments was minor and not significant in the bootstrap randomization test, which shows no significant difference between the temperature treatments (p = 0.1114, Fig. 8 B). The decrease of Δ-bleaching score in RE corals was larger and significantly different in the bootstrap randomization test (p = 0.0431, Fig. 8 C).

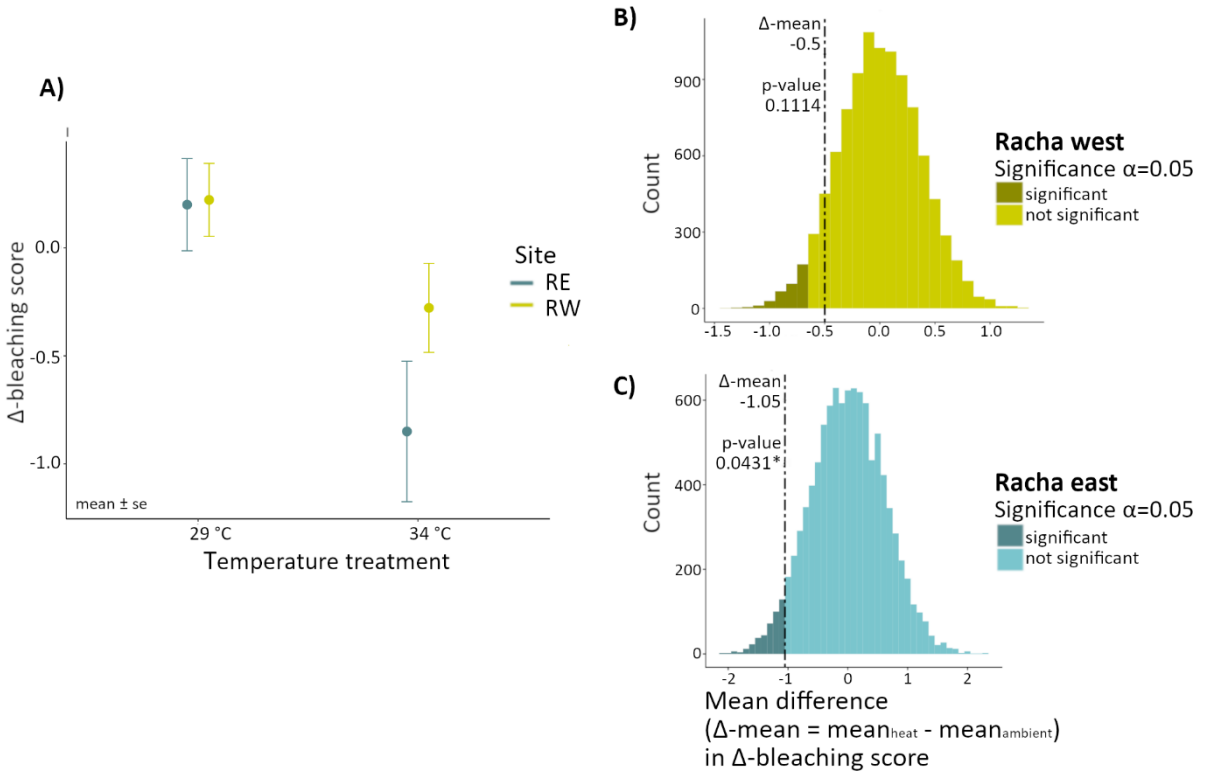

Figure 8 Δ-bleaching score (change of bleaching score over time) from the heat stress assay comparing responses between corals from Racha west (RW) and east (RE). (A) Means of Δ-bleaching score of *Pocillopora spp.* fragments grouped by heat stress treatment (heat = 34 °C, ambient = 29 °C) and site RE or RW. The bleaching score was recorded by categorizing fragments on a scale from 1 (completely bleached) to 6 (healthy). Points depict means ± standard error (se). (B & C) A randomization test (10,000 bootstraps) was performed for the mean difference in Δ-bleaching score between the temperature treatments (ambient and heat) for each RW and RE corals (Δ-mean (RE or RW) = mean Δ-bleaching score $_{heat}$ – mean Δ-bleaching score $_{ambient}$). Given an alpha of 0.05, *P*-values were calculated as values smaller than the true Δ-mean (dashed line) divided by 10,000 bootstraps.

4.1.2 Photosynthetic efficiency

Coral fragments from RW displayed a significantly higher photosynthetic performance compared to their conspecifics from RE after the heat treatment. Coral fragments from RE showed a strong decrease in effective quantum yield (ΔF/Fm) over the course of the heat treatment, dropping from 0.669 ± 0.001 at 0 h to 0.521 ± 0.08 at 24 h (Fig. 9). In contrast, fragments from RW were able to maintain similar levels than the fragments from the ambient treatments (0.658 ± 0.006 at 0 h and 0.657 ± 0.007 at 24 h, Fig. 9). Additionally, all fragments maintained stable ΔF/Fm rates in ambient conditions of over the course of the experiment (RE: 0.675 ± 0.001 at 0 h and 0.679 ± 0.003 at 24 h , RW: 0.665 ± 0.007 at 0h and 0.664 ± 0.01 at 24 h; Fig. 9).

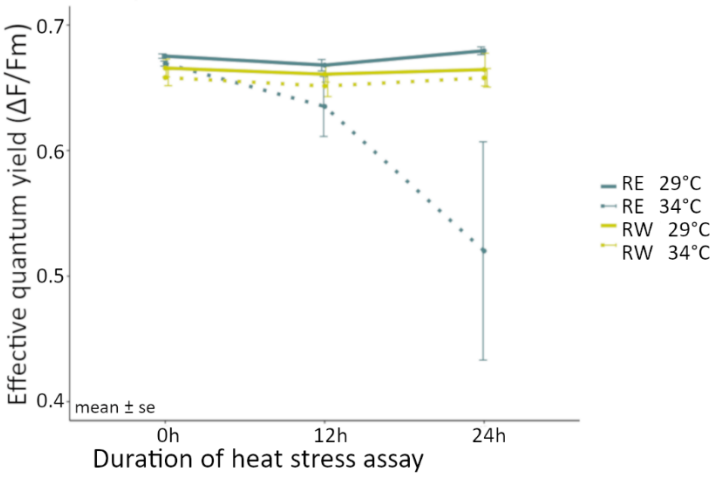

Figure 9 Photosynthetic efficiency Φ PSII as effective quantum yield (ΔF/Fm) over the duration of the heat sress assay (treatment of either ambient = 29°C or heat = 34°C) of *Pocillopora sp*. fragments from Racha east (RE) and Racha west (RW). Points depict means ± standard error (se).

A significant interaction was identified for Δ-effective quantum yield between the site and the treatment (t = -3.824, *p* = 0.000131, Table 5), but not for site only (t = 1.141, *p* = 0.254, Table 5). A post-hoc test highlighted a significant difference in Δ-effective quantum yield between temperature treatments in RE fragments (z = -5.991, p < 0.0001, Table 6, Fig. 10), whereas Δ-effective quantum yield was not significantly different in RW corals (z = -0.412, *p* = 0.976, Table 6, Fig. 10).

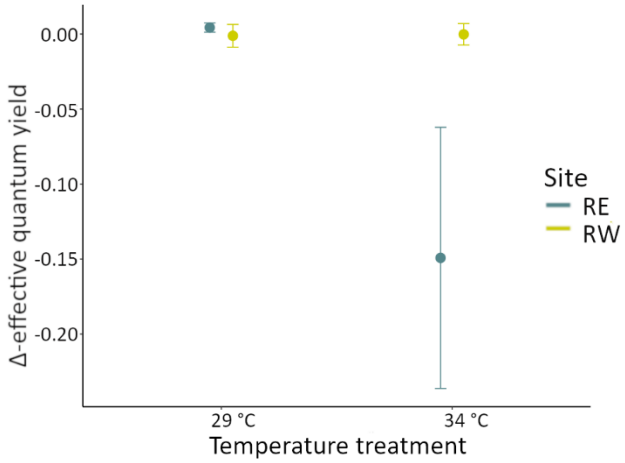

Figure 10 Δ-effective quantum yield of *Pocillopora spp.* per temperature treatment (ambient = 29 °C and heat = 34 °C) per site Racha east (RE) and west (RW). The change of effective quantum yield over the course of the experiment (Δ-effective quantum yield) was calculated by subtracting the final value at 24 h from the initial value at 0 h. Points depict means ± standard error (se).

Table 5 Generalized linear mixed effect model for photosynthetic efficiency Φ PSII as Δ-effective quantum yield of *Pocillopora spp*. fragments from Racha east and west of the heat stress assay. Std. Error indicates standard error. Significant terms are highlighted in bold.

Response variable	Fixed effects	Estimate	Std. Error	t value	*Pr*(>|z|)
Δ-effective quantum yield	(Intercept)	-4.779	0.341	-14.022	**< 0.001 *****
	Site	0.564	0.495	1.141	0.254
	Heat stress treatment	2.888	0.482	5.991	**< 0.001 *****
	Site x heat stress treatment	-2.679	0.701	-3.824	**< 0.001 *****

Table 6 Tukey multiple pairwise post-hoc test for fitted generalized linear mixed effect model for photosynthetic efficiency Φ PSII as Δ-effective quantum yield of *Pocillopora sp*p. fragments. Std. Error indicates standard error. Significant terms are highlighted in bold.

Response variable	Contrasts	Estimate	Std. Error	z ratio	p value
Δ-effective quantum yield	RE ambient - RW ambient	-0.565	0,495	-1,1410	0.664
	RE ambient - RE heat	-2.888	0.482	-5,9910	**< 0.001 *****
	RE ambient - RW heat	-0.774	0.495	-1,5640	0.399
	RW ambient - RE heat	-2.323	0.495	-4,6910	**< 0.001 *****
	RW ambient - RW heat	-0.209	0.508	-0,4120	0.976
	RE heat - RW heat	2.114	0.495	4,2680	**< 0.001 *****

4.2 Microbiome transplantation experiment

4.2.1 Bleaching score

Bleaching scores were measured to assess the change in photosymbiont density of coral fragments during the inoculation experiment. Overall, bleaching scores in the inoculation and control treatment remained similar between 0 h and 24 h (Fig. 11). The inoculation treatment had no effect on the Δ-bleaching score (p = 0.5438, Fig. 12).

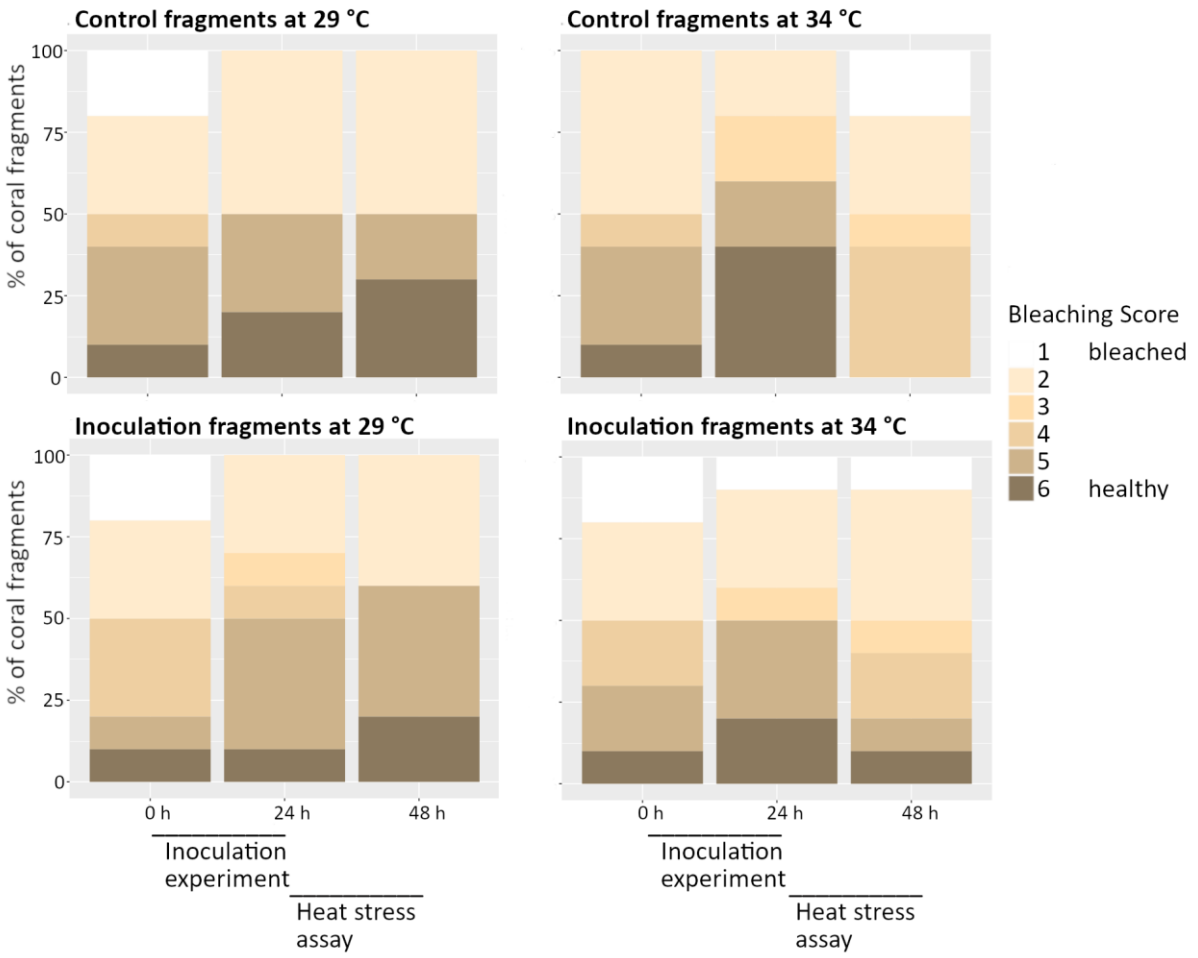

Figure 11 Bleaching scores of *Pocillopora spp.* fragments during the microbiome transplantation experiment. The bleaching score was recorded at the beginning at 0 h, after 24 h and at the end of the experiment after 48 h by categorizing coral fragments on a scale from 1 (bleached) to 6 (healthy). The inoculation experiment was carried out within the first 24 h and was followed by the heat stress assay until 48 h.

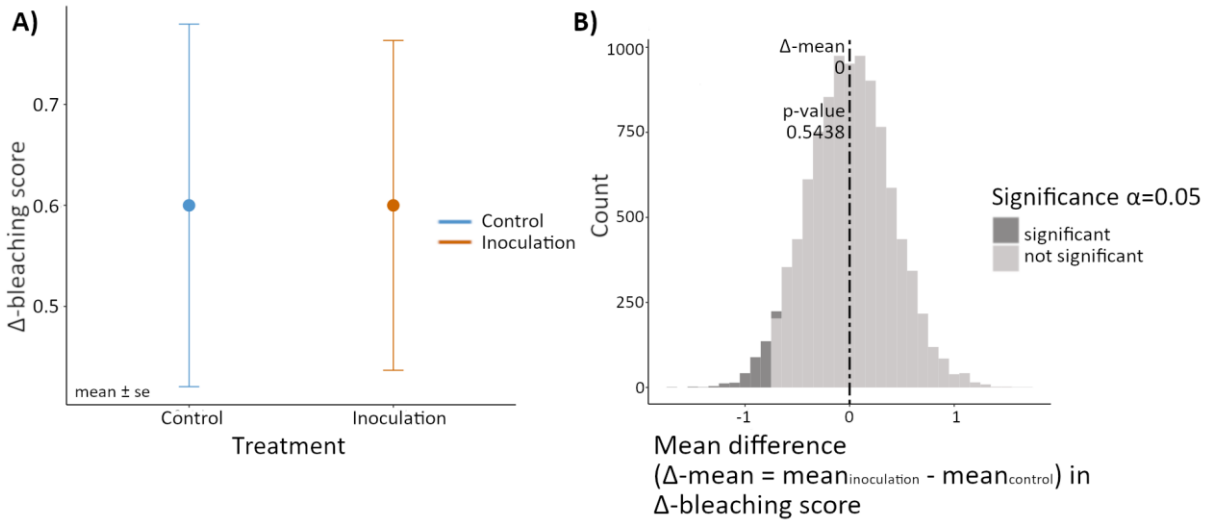

Figure 12 Δ-bleaching score (i.e. the change in bleaching score over time) from the inoculation experiment within the first 24 h of the microbiome transplantation experiment. (A) Means of Δ-bleaching score of *Pocillopora spp.* fragments grouped by treatment (inoculation and control). The bleaching score was recorded by categorizing fragments on a scale from 1 (completely bleached) to 6 (healthy). Points depict means ± standard error (se). (B) A randomization test (10,000 bootstraps) was performed for the mean difference in Δ-bleaching score between the treatments (Δ-mean inoculation treatment = mean Δ-bleaching score $_{inoculation}$ – mean Δ-bleaching score $_{control}$). Given an alpha of 0.05, *P*-values were calculated as values smaller than the true Δ-mean (dashed line) divided by 10,000 bootstraps.

The subsequent heat stress assay (from 24 h to 48 h of the experiment) aimed at comparing stress responses of previously inoculated coral fragments (I) and the control group (C) subjected to a heat (H) and ambient (A) temperature treatments (resulting in four sample groups IA, IH, CA and CH). Results show that CH fragments were the only sample group displaying a significant decrease in bleaching score (Fig. 13 A). Importantly, signs of stress can be observed for both, CH as well as IH treated fragments, with a certain decrease in Δ-bleaching observed in both groups (Fig. 13 A). However, this decrease was only significant for CH fragments since they differed significantly in Δ-bleaching score from CA fragments (p = 0.0026, Fig. 13 C). In contrast, the groups IA and CA showed no change in bleaching score over the course of the experiment (Fig. 13 B).

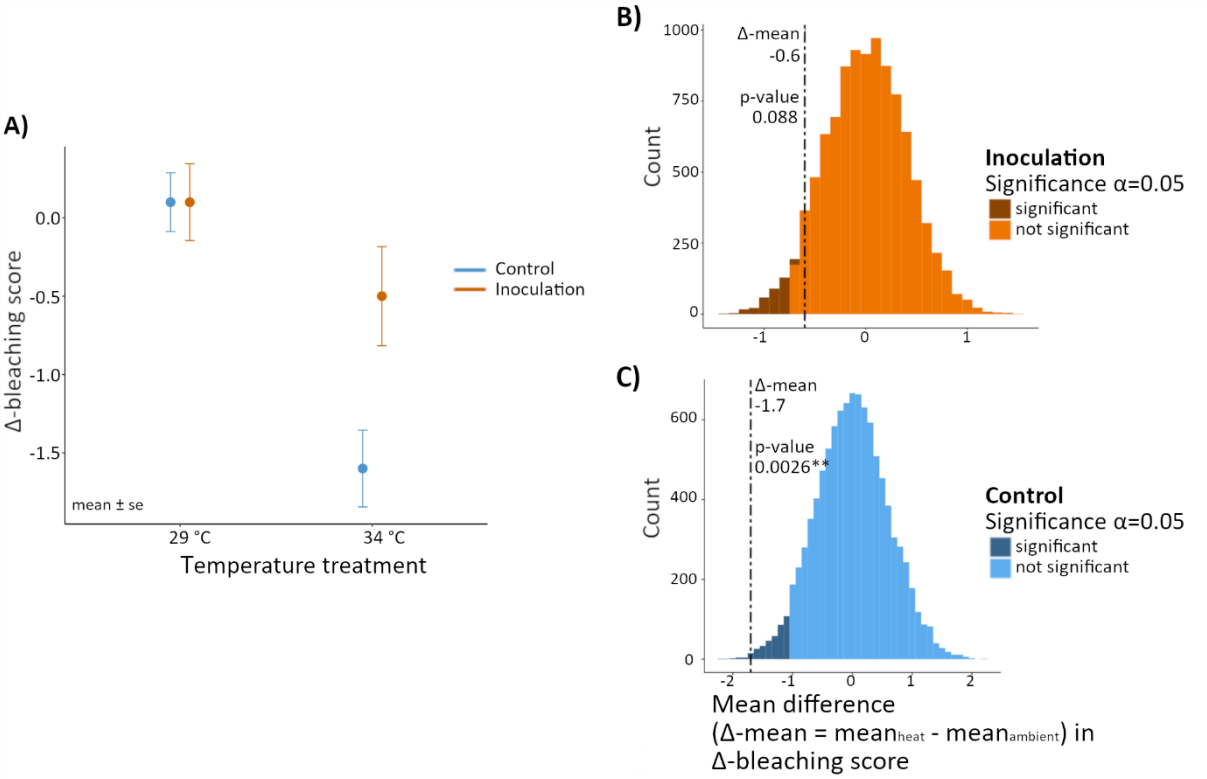

Figure 13 Δ-bleaching score (i.e. the change in bleaching score over time) from the heat stress assay within the second 24 h of the microbiome transplantation experiment. (A) Means of Δ-bleaching score of *Pocillopora spp.* fragments were grouped by inoculation treatment (inoculate and control) and temperature treatments (heat = 34 °C and ambient = 29 °C). The bleaching score was recorded by categorizing fragments on a scale from 1 (completely bleached) to 6 (healthy). Points depict means ± standard error (se). (B & C) A randomization test (10,000 bootstraps) was performed for the mean difference in Δ-bleaching score between the temperature treatments (ambient and heat) for each coral fragments treated with the inoculation or the control (Δ-mean (I or C) = mean Δ-bleaching score $_{heat}$ – mean Δ-bleaching score $_{ambient}$). Given an alpha of 0.05, *P*-values were calculated as values smaller than the true Δ-mean (dashed line) divided by 10,000 bootstraps.

4.2.2 Photosynthetic efficiency

We did not observe any apparent changes over time in ΔF/Fm values visible between inoculation and control treatments during the inoculation experiment (inoculate: 0.672 ± 0.004 at 0 h and 0.664 ± 0.004 at 12 h, control: -0.01 ± 0.004; Fig. 14). Moreover, there was no significant effect of the inoculation and control treatment on Δ-effective quantum yield (p = 0.5368, Table 7, Fig. 15).

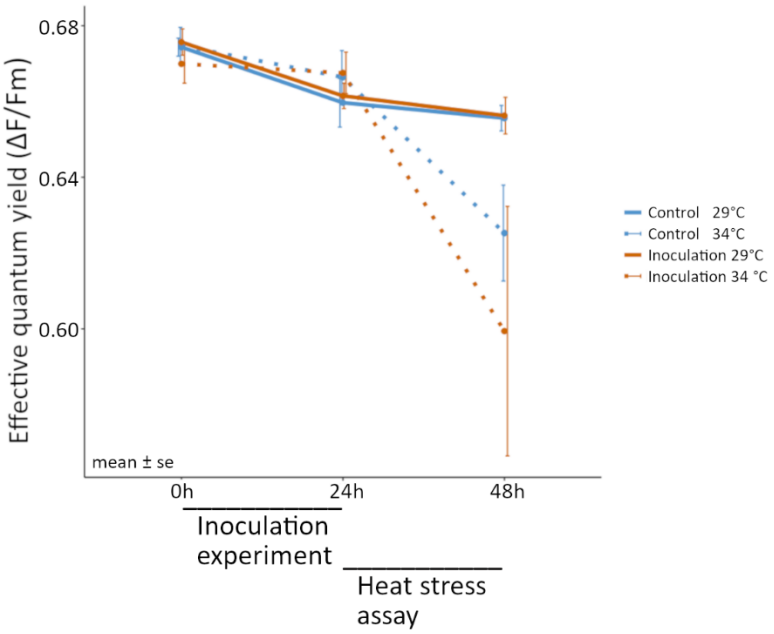

Figure 14 Photosynthetic efficiency Φ PSII as effective quantum yield (ΔF/Fm) of *Pocillopora spp*. over the duration of the experiment. The inoculation experiment (inoculation and control treatment) was carried out within the first 24 h, following by the heat stress assay (heat = 34 °C and ambient = 29 °C) until 48 h. Points depict means ± standard error (se).

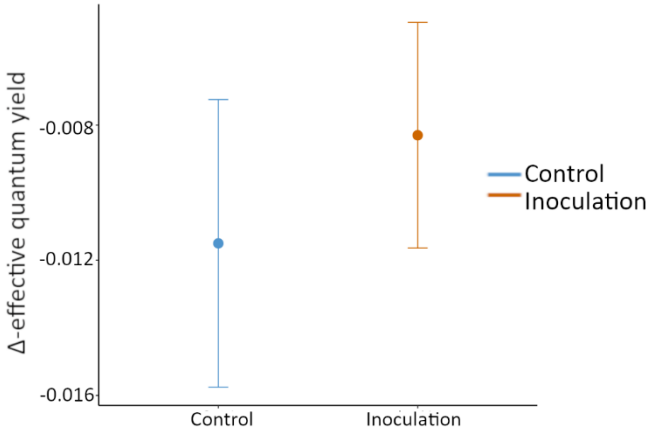

Figure 15 Δ-effective quantum yield of *Pocillopora spp*. fragments from the inoculation experiment as part of the microbiome transplantation experiment. Fragments were exposed to either inoculation or control treatment within the first 24 h of the microbiome transplantation experiment. Δ-effective quantum yield was estimated by subtracting the final value at 24 h from the initial value at 0 h. Points depict means ± standard error (se).

Table 7 Linear mixed effect model for photosynthetic efficiency ΦPSII as Δ-effective quantum yield of *Pocillopora spp*. fragments during the inoculation experiment as part of the microbiome transplantation experiment. Significant terms are highlighted in bold.

Response variable	Fixed effects	denDF	F	P
Δ-effective quantum yield	(Intercept)	16	21.78	**< 0.001 *****
	Inoculation Treatment	2	0.54	0.537

Overall, effective quantum yield decreased in both inoculation and control treatments when fragments were exposed to heat (inoculate: from 0.667 ± 0.005 at 24 h and 0.599 ± 0.03 at 48 h, control: from 0.666 ± 0.007 at 24 h to 0.625 ± 0.01 at 48 h, Fig. 14).

GLMM analysis underlined that both decreases in Δ-effective quantum yield were significant (t = 2.52, *p* = 0.0117, Table 8, Fig. 16) and a post-hoc test revealed that there was no statistical difference between the decrease in both treatments (z = -0.982, p = 0.7597, Table 9). Under ambient conditions, control and inoculation fragments showed no difference in effective quantum yield (control: 0.659 ± 0.006 at 24 h and 0.655 ± 0.003 at 48 h, inoculate: 0.661 ± 0.003 at 24 h and 0.656 ± 0.004, Fig. 14). GLMM and post-hoc analysis also displayed no difference in Δ-effective quantum yield between control and inoculation treatment during the ambient treatment (z = 0.511, *p* = 0.9566, Table 9).

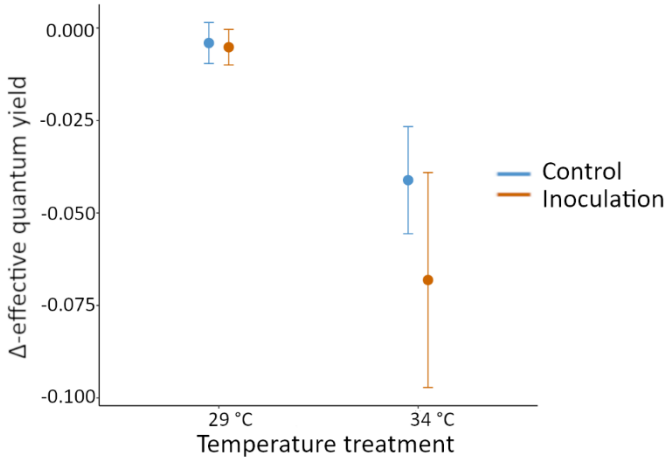

Figure 16 Δ-effective quantum yield of *Pocillopora spp*. fragments from the heat stress assay as part of the microbiome transplantation experiment. Inoculated and control coral fragments were exposed to the respective heat stress treatment (ambient = 29 °C and heat = 34 °C) from 24 h until 48 h. Points depict means ± standard error (se).

Table 8 Generalized linear mixed effect model for Δ-effective quantum yield over the duration of the heat stress assay as part of the microbiome transplantation experiment of control and inoculated fragments of *Pocillopora spp*. Std. Error indicates standard error. Significant terms are highlighted in bold.

Response variable	Fixed effects	Estimate	Std. Error	t value	Pr(>|z|)
Δ-effective quantum yield	(Intercept)	-4.486	0.363	-12.345	**< 0.001 *** **
	Inoculation treatment	-0.262	0.514	-0.511	0.610
	Heat stress treatment	1.295	0.514	2.520	**< 0.05 ***
	Inoculation treatment x heat stress treatment	0.767	0.727	1.055	0.291

Table 9 Tukey multiple pairwise post-hoc test of fitted generalized linear mixed effect model for Δ-effective quantum yield over the duration of the heat stress assay of *Pocillopora spp*. fragments. Std. Error indicates standard error. Significant terms are highlighted in bold.

Response variable	Contrasts	Estimate	Std. Error	z ratio	*P* value
Δ-effective quantum yield	Control ambient – Inoculation ambient	0.262	0.514	0,511	0.9566
	Control ambient – Control heat	-1.295	0.514	-2.520	0.0596
	Control ambient – Inoculation heat	-1.799	0.514	-3.502	**< 0.01 ** **
	Inoculation ambient – Control heat	-1.557	0.514	-3.031	**< 0.05 ***
	Inoculation ambient – Inoculation heat	-2.062	0.514	-4.013	**< 0.001 *** **
	Control heat – Inoculation heat	-0.505	0.514	-0.982	0.7597

4.2.3 Preliminary bacterial community analysis

In total, 114,998 16S rRNA sequences were retained after Illumina Miseq sequencing, merging and sequence editing. Subsampling to 4423 reads per sample eliminated 31 samples (including all seawater samples) containing 1 to 772 sequences. Consequently, this data set did not allow further analysis of bacterial communities present in seawater used throughout the experiment. The remaining 26 samples were unevenly distributed among sample groups (recipient fragments before inoculation: 12 samples/colonies; inoculate recipient fragments after inoculation: 5; control recipient fragments after inoculation: 7; donor inoculate: 2; Table 1; Table S2). Clustering resulted in 2549 OTUs at 97% a similarity cut-off.

We provide a preliminary characterization of the microbial community composition and structure of *Pocillopora spp*. fragments used in the microbiome transplantation experiment. In general, microbiomes of *Pocillopora spp.* were dominated by the families Rhodobacteraceae (15%), Flavobacteriaceae (6%), Cyclobacteriaceae (4%), Saprospiraceae (4%), Alteromonadaceae (4%), and Pseudoalteromonadaceae (3%; average relative percentages across samples, Fig. S1). We did not find any bacterial family that dominated the communities by more than 50%. Estimated species richness (Chao 1 index) was highest and significantly different in the donor inoculate sample compared to the other samples of the inoculation experiment (Fig. 17 A; t = 3.56, $p < 0.001$, Table 10). Estimated species richness (Shannon index) did not differ significantly between samples (Fig. 17; Table 10).

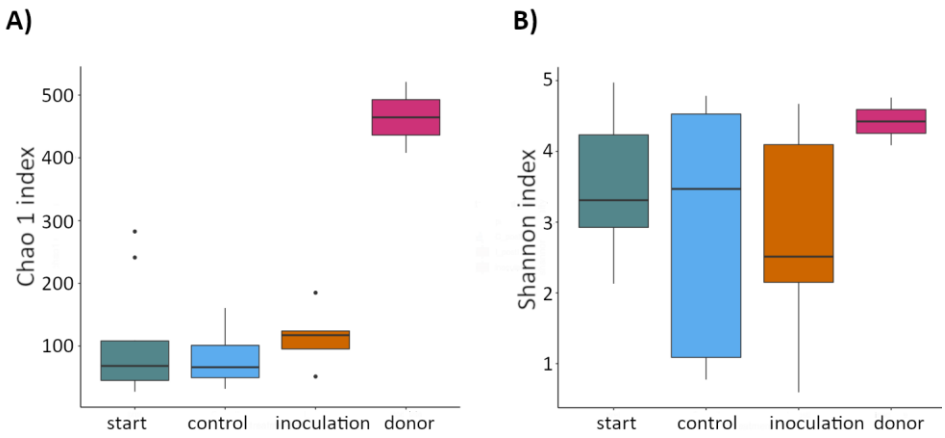

Figure 17 α-diversity comparison between recipient fragments before inoculation (= start), control recipient fragments after inoculation (= control), inoculation recipient fragments after inoculation (= inoculation), and donor inoculate (= donor) of *Pocillopora spp*. Displayed are A) estimated species richness (Chao 1 Index) and B) estimated species diversity (Shannon Index).

Table 10 Generalized linear mixed effect model for each α-diversity index (Chao 1 = estimated species richness and Shannon index = estimated species diversity) of bacterial communities of *Pocillopora spp*. fragments from the inoculation experiment as part of the microbiome transplantation experiment. Significant terms are highlighted in bold. Std. error indicates standard error.

Response variable	Fixed effects	Estimate	Std. Error	t value	Pr(>|z|)
Chao 1 Index	(Intercept)	4.580	0.166	27.641	**< 0.001 *****
	Control recipient coral fragments	-0.196	0.273	-0.716	0.473
	Inoculate recipient coral fragments	0.163	0.305	0.533	0.593
	Donor inoculate	1.561	0.438	3.561	**< 0.001 *****
Shannon Index	(Intercept)	1.241	0.137	9.006	**< 0.001 *****
	Control recipient coral fragments	-0.178	0.227	-0.786	0.432
	Inoculate recipient coral fragments	-0.211	0.254	-0.827	0.408
	Donor inoculate	0.245	0.365	0.673	0.501

Microbial community composition at the OTU level visualized by a two-dimensional principle coordinate analysis (PcoA) based on the Jaccard index dissimilarity matrix cluster significantly apart clustered between donor homogenate and recipient fragment samples (Fig. S2; ANOSIM, R = 0.29, p = 0.0036). Clustering between recipient sample groups and start recipient fragment samples was very minor with an overlap of control recipients and inoculate recipient samples (Fig. S2).

4.2.4 First identification of potentially transplanted bacterial taxa

Subsequently, we present a first analysis towards identifying candidate bacterial taxa that have been potentially transplanted from the donor corals using a fresh tissue homogenate applied to recipient corals as inoculate. A Venn diagram shows the unique, shared, and ubiquitous OTUs across treatments in coral samples and the donor inoculate (Fig. 18). To identify whether and which OTUs were potentially transplanted by inoculation, the shared OTUs between the donor homogenate samples and recipient coral fragments were were extracted (Table 11). More information on sequence identity from the SILVA (release n. 132; Quast 2013) and GenBank (NBCI database) are displayed in the same table. In total, 9 OTUs were shared between the two sample groups (Fig. 18). These candidate OTUs were assigned to the bacterial families Spongiibacteraceae, Micavibrionaceae, Geminicoccaceae, Bdellovibrionaceae, Rubinisphaeraceae, Cryomorphaceae and Alteromonadaceae (Table 11). They were found in relatively low abundances of <0.3 % of total reads per sample and in 1-2 samples of all recipient samples (N=5).

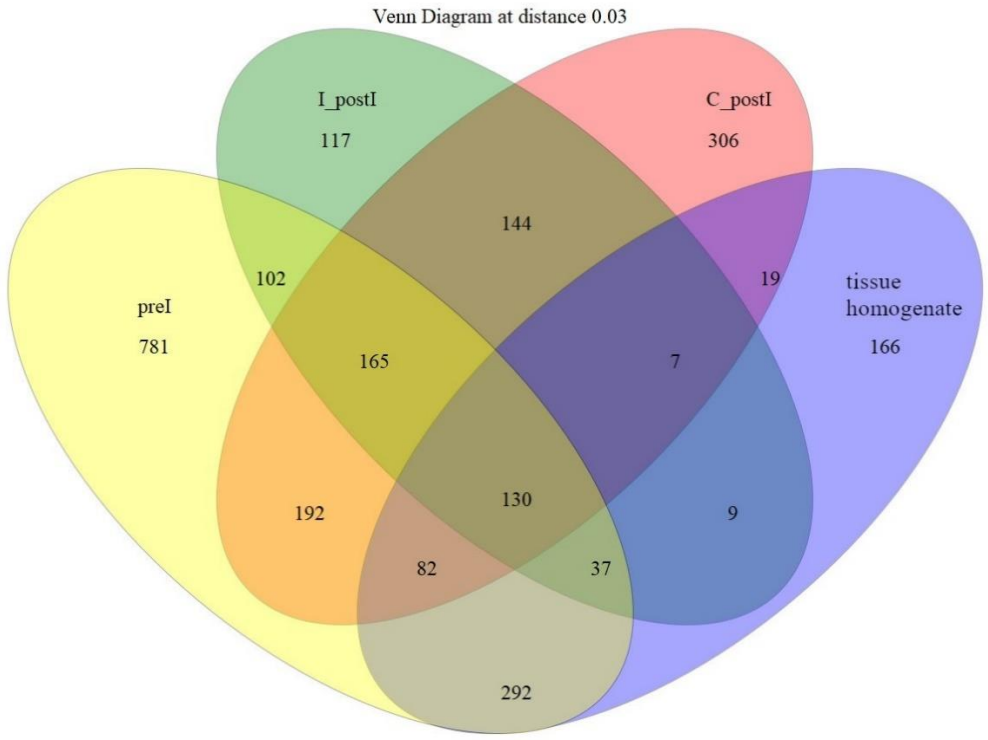

Figure 18 Venn diagram displaying absolute numbers of unique, shared, and ubiquitous OTUs (97% similarity cut-off) in different treatment groups (pre_I = recipient coral fragments before inoculation, C_postI = control recipient coral fragments after inoculation, I_postI = inoculate recipient coral fragments after inoculation) of *Pocillopora spp*. and the inoculate, a homogenate of fresh donor tissue (= tissue homogenate) from the microbiome transplantation experiment.

Table 11 Annotation of OTUs exclusively found in the donor inoculate and inoculate recipient coral fragments of *Pocillopora spp*. Assignment of OTUs was conducted with SILVA (release n. 132) and BLASTn (GenBank, NCBI) based on representative sequences per OTU generated in MOTHUR (version 1.39.5).

OTU	SILVA taxonomy	Nearest relative BLASTn result [accession n.]	*E*-value	Query cover (%)
Otu01043	Spongiibacteraceae, clade BD1-7	*Zhongshania aliphaticivorans* strain SM-2 [NR_126306.1]	$3.0e^{-116}$	100
Otu01662	Micavibrionaceae, uncultured	*Thalassospira mesophila* strain MBE#74 [NR_114387.1]	$2.0e^{-77}$	100
Otu01693	Geminicoccaceae, unclassified	*Geminicoccus roseus* strain D2-3 [NR_042567.1]	$4.0e^{-90}$	75
Otu01763	Bdellovibrionaceae, Bdellovibrio	*Bdellovibrio bacteriovorus* strain HD100 [NR_027553.1]	$2.0e^{-85}$	100
Otu02291	Rubinisphaeraceae, Planctomicrobium	*Bythopirellula goksoyri* strain Pr1d [NR_118636.1]	$4.0e^{-65}$	81
Otu02554	Cryomorphaceae, uncultured	*Vicingus serpentipes* strain ANORD5 [NR_159281.1]	$3.0e^{-111}$	100
Otu03123	Alteromonadaceae, uncultured	*Catenovulum maritimum* strain Q1 [NR_146038.1]	$2.0e^{-108}$	100
Otu03797	Bdellovibrionaceae, Bdellovibrio	*Bdellovibrio bacteriovorus* strain HD100 [NR_027553.1]	$1.0e^{-80}$	71

5. Discussion

In recent years, elevated sea surface temperatures caused by global climate change have led to major declines in coral reef ecosystems which are mainly due to mass coral bleaching events of increasing frequency and severity (Hughes et al. 2018). Therefore, interventions are needed to prevent further loss of reef-building corals and to restore these valuable ecosystems (van Oppen et al. 2015). Nowadays, coral reef restoration programs aim at returning a habitat to a pre-disturbance state by growing coral fragments from the original stock in *ex situ* or *in situ* coral nurseries (e.g., floating *in situ* nurseries) and then transplanting the farmed fragments onto the degraded reefs (Rinkevich 2014). However, the original stock of coral colonies might not be resilient against further ongoing environmental changes and is therefore likely to endure losses again due to persistent stressors (van Oppen et al. 2015). The novel idea of assisted evolution aims at accelerating evolutionary processes to increase the resilience of corals, which is promising to provide a long-term sustainable solution for coral reef restoration that could be applied aside of the important task of tackling the causes of climate change such as curbing greenhouse gas emissions. Since it is suggested that coral heat tolerance could be conferred by the bacterial microbiome (Ziegler et al. 2017), i.e. coral-associated prokaryotes, transplantation of a certain beneficial bacterial taxon or consortium could be a powerful method of assisted evolution to mitigate coral bleaching. Targeting coral phenotypes, which are already more heat-tolerant than others, is a promising strategy for sourcing beneficial microbiome functions, since it allows for the omission of time-consuming and expensive multigenerational coral selection experiments. The method of using a fresh tissue homogenate as inoculate to transplant bacteria between corals further bypasses time consuming and expensive microbiological cultivation. Since most field-based reef restoration programs cannot afford elaborate facilities and tools, they are seeking fast and affordable but auspicious methods. Consequently, such a cost-effective approach could find application in future large-scale coral reef restoration programs.

5.1 Corals exposed to long-amplitude internal waves (LAIW) show a higher heat tolerance

The first part of this study aimed at identifying resilient, in particular heat-tolerant, corals as a source of potentially beneficial bacterial communities. In this regard, the study made use of a unique coral reef system in the Andaman Sea in Thailand. Here, we

explored corals which live in a high-variability environment comprising strong temperature, nutrients, and pH fluctuations due to the impact of large-amplitude internal waves (LAIW) on the shallow reefs (Jantzen et al. 2012). These coral populations are hypothesized to exhibit a high heat tolerance. Such high fluctuations of environmental conditions, especially temperature, exert strong selection pressures and are therefore assumed to increase the resilience of corals through acclimation (Oliver and Palumbi 2011; Camp et al. 2018). This has been shown to be true for several corals in coral reef habitats such as tidal pools (Palumbi et al. 2014). In this study, we conducted short-term heat stress experiments in order to determine the heat tolerance of high variability (LAIW) exposed corals from Racha Island west shore (RW) and compared responses to conspecifics from a sheltered reef site of Racha Island east shore (RE). Overall, LAIW exposed corals from RW were able to maintain their physiological performance under a short but acute heat stress, compared to RE corals. Heat stress in RE corals led to the expulsion of dinoflagellate photosymbionts and lowered their photosynthetic performance, whereas most RW coral colonies were able to widely maintain levels of symbiont density and their photosynthetic performance.

The higher heat tolerance of LAIW exposed RW corals could underlie various factors. One aspect that could influence better performance under heat stress conditions may be a specific clade of the photosymbiont dinoflagellate (Rowan 2004). Therefore, difference in heat-tolerance between RW and RE corals could be due to hosting different thermally tolerant photosymbiotic dinoflagellates (Berkelmans and Van Oppen 2006). Based on a previous study of *Porites lutea* from the same island system in Thailand (Similan Island) which showed that the ITS-2 photosymbiont type did not differ between east and west shore reefs (Buerger et al. 2015), it could be hypothesized that the photosymbiont type also did not differ in *Pocillopora spp*. from our study sites. However, the specific photosymbiont type of *Pocillopora spp*. from our study sites needs to be tested since different species can differ in their photosymbiont clade, as previously shown for *Pocillopora verrucosa* and *Porites lutea* (Ziegler et al. 2015). Another component which plays an essential role in defining heat tolerance of coral holobionts is the coral host. For instance, rich energy reserves (i.e. lipid, protein, carbohydrate content in tissues) have been shown to be an essential element of coral heat tolerance, enabling the maintenance of metabolic processes during stress (Grottoli et al. 2004; Anthony et al. 2009). This finding was also supported by an earlier study on LAIW exposed colonies of *P. lutea* showing higher protein contents (Roder et

al. 2011), but needs to be further investigated for *Pocillopora. spp.* from RW and RE. Finally, the third player of the coral holobiont, the bacterial microbiome, could be contributing to a better performance under heat stress. It has been suggested that specific members of the microbiome may be involved in tuning the physiological responses to heat stress as it has been shown that coral microbiome composition aligns with heat tolerance of the coral host (Ziegler et al. 2017). One microbial process that could be involved in helping the coral tolerate heat stress could be the degradation of reactive oxygen species (ROS). These compounds are produced during stress inside the host and photosymbiotic dinoflagellate cells and are suggested to trigger coral bleaching (Tchernov et al. 2004; Smith et al. 2005). Another potentially beneficial microbial process could be antagonistic activity against coral pathogens that are known to trigger bleaching, such as *Vibrio coralliilyticus* (Ben-Haim et al. 2003). Based on previously mentioned microbiome studies, we hypothesize that heat-tolerant corals from RW could host bacterial taxa and communities that are beneficial for the coral holobiont under heat stress. Nevertheless, the specific microbiome characteristics associated with heat-tolerant RW corals need to be further identified, which was beyond the scope of this study.

5.2 Tissue homogenate mitigates coral bleaching signs under acute short-term heat stress

The main objective of the second part of this study was to transfer the microbiome components from the bleaching resilient corals to heat-sensitive corals, hypothesizing that their microbiome might help to improve heat tolerance of heat-sensitive corals. For this purpose, a tissue homogenate was produced from donor RW fragments to be inoculated onto recipient RE corals. Overall, the bleaching response of RE corals to acute short-term heat stress was mitigated by inoculation. Inoculated RE corals did not bleach significantly under short term heat stress, which was comparable to the response quantified for RW donor corals (Table 12). In contrast, the control RE group responded with a significant decrease in bleaching score, similar to the response of RE corals previously shown (Table 12). Interestingly, photosynthetic efficiency of dinoflagellate photosymbionts was decreased under heat in both inoculation and control recipient fragments, which indicated that photosymbiont stress could not be mitigated by the inoculation treatment. These results of the decrease in photosymbiont performance could suggest that the inoculated coral holobiont was able to counteract their expulsion and buffer bleaching responses to heat stress. Therefore, stress factors

that usually trigger the expulsion of the photosymbionts could have been suppressed by bacterial consortia or other components found in the inoculate treatment, which needs to be further analysed.

Table 12 Summary of the results of stress responses of *Pocillopora spp.* fragments (RW = coral fragments originating from Racha Island west, RE = coral fragments from Racha Island east, Inoculated RE = RE coral fragments inoculated with a tissue homogenate from RW corals, Control RE = RE coral fragments treated with filtered sea-water) to heat stress (heat treatment) or inoculation. Stress responses were measured as bleaching score and effective quantum yield (ΔF/Fm).

Sample group	RW	RE	Inoculated RE	Control RE
Decrease of bleaching score after heat treatment	**+**	++	**+**	++
Decrease of ΔF/Fm after heat treatment	**0**	++	++	++
Decrease of bleaching score after inoculation treatment only	n.a.	n.a.	0	0
Decrease of ΔF/Fm after inoculation treatment only	n.a.	n.a.	0	0

5.3 Preliminary mining for potentially transplanted bacterial taxa

We analysed the 16S bacterial community composition in order to investigate if the mitigated bleaching response of inoculated recipient corals was associated with major bacterial community restructuring. Results showed that inoculation did not cause any major restructuring in microbiome community composition. First, there were no observed significant changes of the species richness and diversity measures of corals in both groups: inoculate recipients and control fragments. Further, clustering of community data in a principal coordinate analysis (PCoA) also displayed that communities did not undergo any major shifts in any of the two groups. Yet, bacterial communities of donor inoculate samples and all recipient coral samples clustered significantly apart, representing a significant difference between bacterial community composition between RW and RE corals. This result might support the hypothesis that RW corals could harbor different bacterial consortia than RE corals which could contribute to their heat tolerance, thus supporting the idea of using RW microbiomes for inoculations.

The most abundant bacterial families found across all coral samples reflected phyla that have been described to be part of the core coral microbiome such as Bacteriodetes (Hernandez-Agreda et al. 2017; identified families i.e. Cryomorphaceae, Flavobacteriaceae, Cyclobacteriaceae, Saprospiraceae). Endozoicomonas, a genus which is often found at highest abundances in the coral microbiomes (Blackall et al.

2015), was also present across all samples. However, relative abundances of Endozoicomonas of the *Pocillopora sp*. samples were much lower than reported for *Porites lutea* samples from the same environment in the Andaman Sea (Pootakham et al. 2018). Endozoicomonas is suggested to play a major role in coral health (Hernandez-Agreda et al. 2017) and was found to be in stable association with *Pocillopora verrucosa* even under heat stress (Pogoreutz 2018).

Next, Venn diagrams and subsequent analysis aimed at identifying new occurrences of specific bacterial taxa found in the inoculated corals, which can be attributed to originating from the donor inoculate. In total, nine OTUs exclusively shared between the donor samples and the inoculation recipient coral fragments were identified, representing potential candidates for transplanted bacterial taxa. One of these candidate OTUs (Otu01043) belonged to the family Spongiibacteraceae which is known to be typically associated with corals, since it was, for instance, found in high abundances in both bleached and non-bleached samples of *Acropora muricata* (Gardner 2019). Additionally, two OTUs (Otu03797, Otu01763) of potentially transplanted candidates were identified as *Bdellovibrio*. This taxon has been found in dominant abundances in the microbiome of the coral *Porites astreoides* and is suggested to be an important bacterial predator within the coral holobiont (Wegley et al. 2007). Bdellovibrios have been described to be parasitic to the bacterium *Vibrio alginolyticus* (Sutton and Besant 2004), a known pathogen that has been associated with mass mortality of the carpet shell clam (Gómez-León 2005). The order of Bacteriovibrionales is in general known to prey on bacteria, especially pathogens (Schoeffield and Williams 1990; Welsh et al. 2016). Indeed, certain pathogens such as *Vibrio coralliilyticus* (Ben-Haim et al. 2003) are known to decrease coral health by triggering bleaching responses. A study of *Pocillopora damicornis* revealed high abundances of *V. coralliilyticus* in bleached fragments and could demonstrate that bleaching and lysis of healthy fragments was caused by an interaction of *V. coralliilyticus* and high seawater temperatures (Ben-haim et al. 2003). Consequently, bacterial antagonistic activity such as the predation of pathogens is suggested to be a beneficial mechanism that could be used for biological control of disease stresses and bleaching of corals (Peixoto et al. 2017). In this regard, a recent study using *Pocillopora damicornis* demonstrated that coral bleaching can be mitigated by inoculating *Pseudoalteromonas sp.* which biologically controlled *V. coralliilyticus* (Rosado et al. 2018). The identification of *Bdellovibrios* exclusively in the donor inoculate and the

inoculated recipient corals of this study leads to the hypothesis that they might have played an indirect role in mitigating bleaching responses through biological control of pathogens triggering the coral bleaching in the experiment. For further assumptions it is neccessary to check for the presence of potential pathogens in the collected samples.

5.4 Lessons from the first coral microbiome transplantation experiment and outlook

This experiment represents a first pilot study which explores the idea of a quick and cost-effective coral microbiome transplantation. In order to provide more evidence and details supporting the interpretation of our preliminary results, additional analyses of the data at hand could be advantageous. First, to assess whether heat tolerance of *Pocillopora spp.* fragments from Racha west (RW) underlies specific photosymbiotic dinoflagellates clades and/or beneficial microbial consortia or taxa, their photosymbiont type and microbiome characteristics need to be identified. Second, results of the microbiome transplantation experiment remain preliminary since important sample sets such as all seawater samples and important replicates were removed due to low read numbers during 16S rRNA sequence quality control and editing. Therefore, it is proposed to repeat DNA-extraction of several problematic samples and repeat sequencing to increase replicate numbers which could provide a more detailed insight into coral microbiome dynamics of the present study. Regarding the identification of bacterial taxa potentially transmitted via inoculation, more analyses beyond the Venn diagrams could reveal more potential candidate OTUs. An analysis that could not only reveal uniquely shared taxa between the inoculate and the inoculated recipients, but rather statistically significant taxa determining the respective sampling groups, could be indicator species analysis (Legendre 2015). Subsequent taxonomy-based functional profiling of the candidate OTUs could also reveal possible bacterial functions that could have contributed to mitigating the stress response of inoculated corals. Additionally, it will be essential to measure cell densities of the tissue homogenate used as inoculate in this study to provide a protocol that can be easily repeated and can be used to test the effects of different concentrations in future experiments. This could be done by passing a fixed sample through a 0.2 µm GTTP filter, staining it with 4′,6-diamidin-2-phenylindol (DAPI) and counting bacterial abundance by epifluorescence microscopy (Glasl et al. 2016).

Our first experimental design and data exploration provide important insights for the design and data analysis of future experiments, as well as new hypotheses which call for a follow up. Especially future studies are required to make this method a valuable and successful tool that can be used in large-scale coral reef restoration efforts. A future experiment that scales up the applied experimental study design by adding more replicate tanks and coral colonies could possibly validate observed trends. Moreover, a study that incorporates multiple inoculations over a longer timescale could enhance bacterial uptake and therefore increase the potential success of the transplantation. In order to better understand underlying mechanisms and potential beneficial functions of transplanted bacterial taxa, microbiological cultivation and meta'omic approaches will be needed. To fully identify and control for other factors that occur in the tissue homogenates and might influence coral heat stress responses, several types of control inoculates, e.g. tissue homogenates treated with antibiotics, will be required for future studies. Since inoculation is conducted using a fresh coral tissue homogenate, rather than pure bacterial isolates, the possibility cannot be excluded that host or bacterial derived proteins and/or metabolites can be transferred and contribute to coral stress response. Given that these controls were not used in the present study, it is possible that other factors aside from the transplanted bacteria might have mitigated coral bleaching responses in the experiment. Furthermore, to improve the understanding of the coral holobiont response to heat stress, more parameters assessing holobiont fitness could be measured in future studies, such as host calcification rates, ratio of photosynthesis to respiration, total lipid contents or density of photosymbiotic dinoflagellates. Most importantly, reef restoration efforts are seeking for approaches that guarantee long-term survival of corals. Therefore, it might be important to apply tissue homogenates comprising beneficial microbes that are able to form stable associations over time and could therefore induce lasting benefits for the coral holobiont (Epstein et al. 2019). Consequently, the stability of newly formed associations of transplanted beneficial microbes and of acquired heat tolerance need to be monitored over longer time scales to ensure long-term benefits for corals under accelerating environmental pressures (Epstein et al. 2019).

5.5 Conclusion

In summary, this study presents a first experimental approach to testing a cost-effective method of coral microbiome transplantation which aims at building coral resilience to

bleaching. By making use of coral from a highly variable reef habitat in the Thai Andaman Sea which exhibited heat tolerance, this study sourced coral microbiome consortia that could potentially confer heat tolerance. Subsequent inoculation using fresh tissue homogenate from these heat-tolerant to heat-sensitive corals led to mitigated bleaching responses under short-term, acute heat stress. Although photosynthetic performance of endosymbiotic dinoflagellates was decreased under heat stress, the inoculated coral holobiont was able to decelerate or prevent the bleaching response. Our first results are preliminary and the role of potentially transplanted bacterial taxa in the alleviated stress response remains hypothetical, making the call for further thorough investigations an urgent one.

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8. Supplementary material

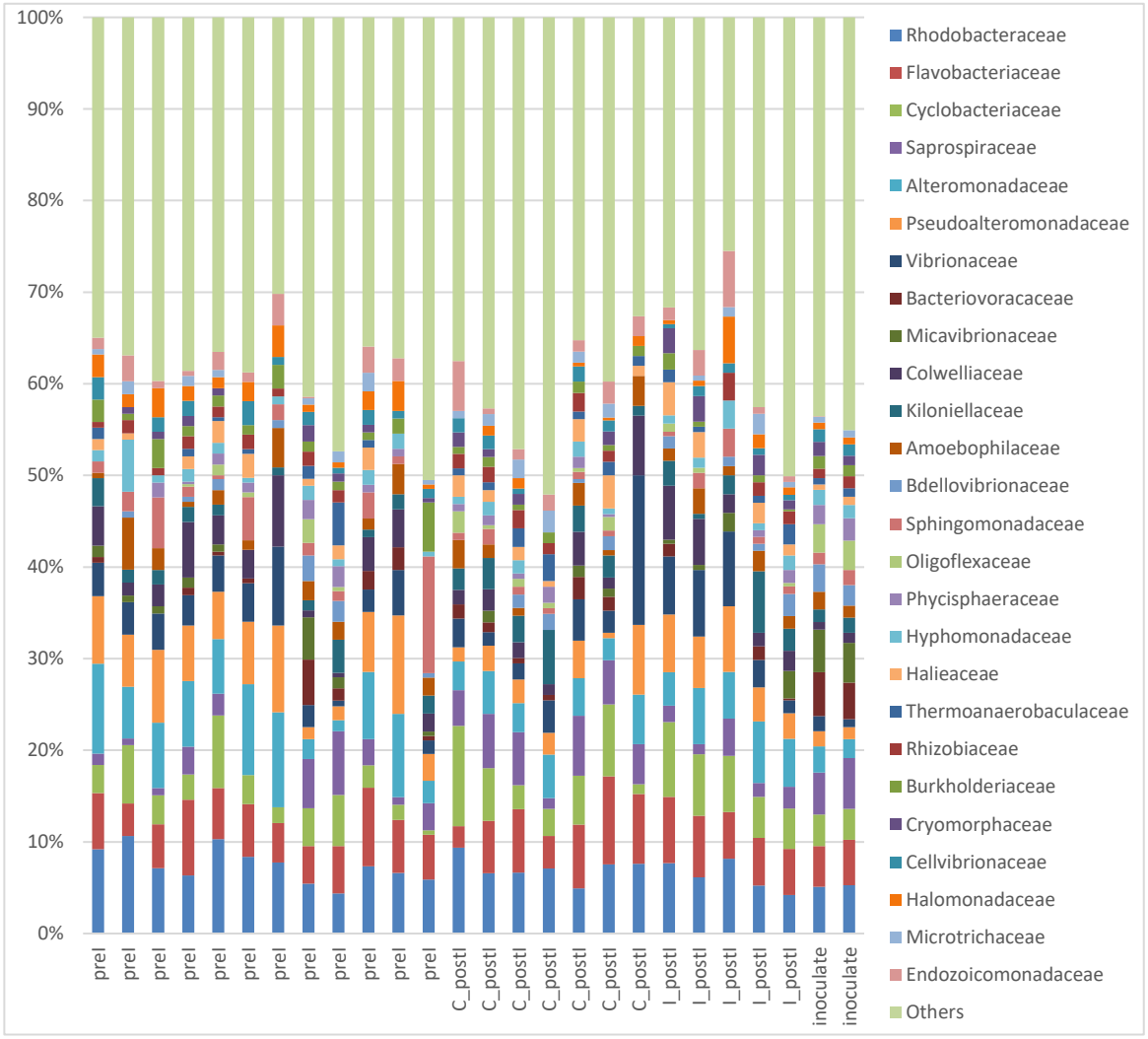

Figure S1 Stack bar plots showing the relative abundances of the 26 most abundant bacterial families contributing either to the microbial assemblages of *Pocillopora spp*. fragments from the various treatments (pre_I = recipient fragments before inoculation , C_postI = control recipient fragments after inoculation, I_postI = inoculate recipient fragments after inoculation) and a donor tissue homogenate (= inoculate) produced from heat-tolerant *Pocillopora spp*. fragments.

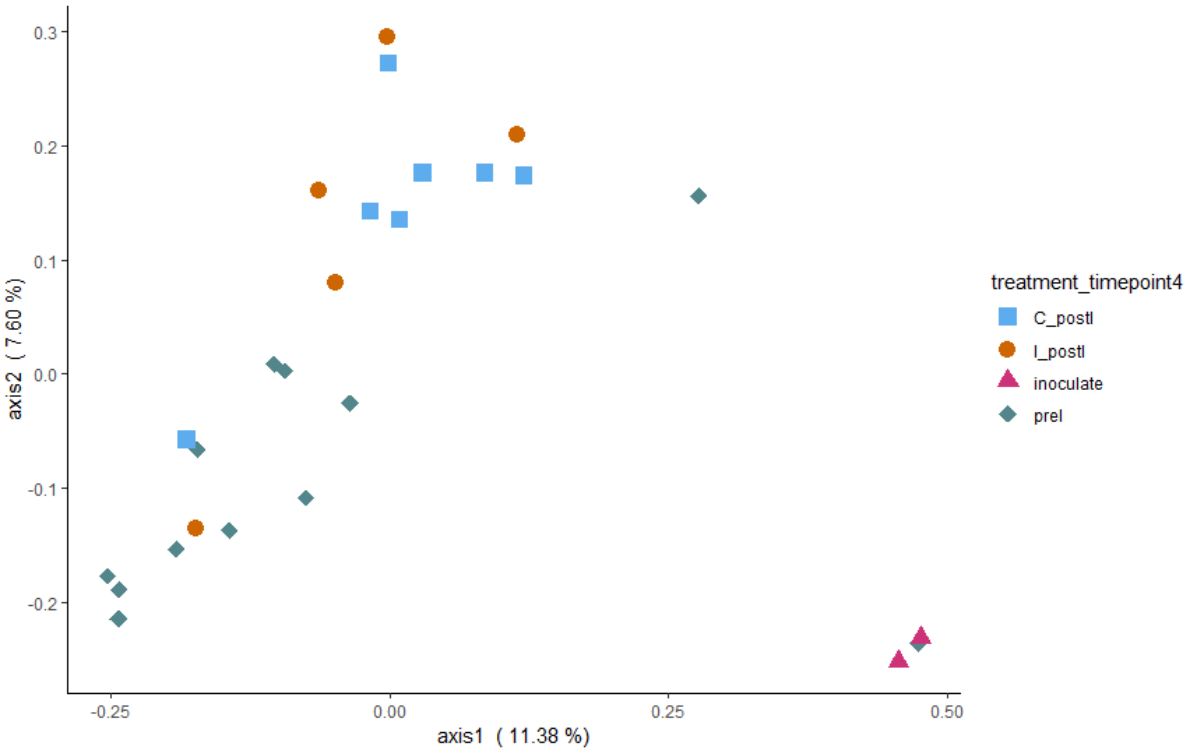

Figure S2 Principal coordinate analysis (PCoA) shows dissimilarities at the OTU level (Jaccard index distance matrix) between samples and displays bacterial community structure of differently treated *Pocillopora spp*. fragments (pre_I = recipient fragments before inoculation , C_postI = control recipient fragments after inoculation, I_postI = inoculate recipient fragments after inoculation) and a donor tissue homogenate (= inoculate).

9. Declaration of authorship

Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Die eingereichte schriftliche Fassung der Arbeit entspricht der auf dem elektronischen Speichermedium.

Weiterhin versichere ich, dass diese Arbeit noch nicht als Abschlussarbeit an anderer Stelle vorgelegen hat.

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